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Evaluation of a mosquito control intervention and recommendations for development of best practice protocols by the Shire of Kalamunda

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Evaluation of a mosquito control intervention and recommendations for development of best practice protocols by the Shire of Kalamunda

This thesis is presented in fulfilment (or partial fulfilment) of the degree of

Master of Public Health

School of Medical and Health Sciences

Edith Cowan University

Kerry Staples

Date of Submission: 11 April 2016

Abstract

The mosquito control program implemented by the Shire of Kalamunda Environmental Health Service has been assessed. Mosquito species and abundance has been evaluated along with an assessment on the current level of pesticide resistance and downstream S-methoprene levels post-treatment. The rate of Ross River virus transmission within the Shire has also been considered, along with the relationship between local species and virus transmission.

Methodology

Floating Emergence Traps were used in 15 storm-water gullies to determine the effectiveness of S-methoprene briquets in prevention of adult mosquito emergence over 124 days. Samples were taken monthly from October 2014 to March 2015. Two treatment methods were assessed, application of briquet using a float, and application without a float. These were compared to untreated control gullies. The productivity of gullies was also assessed. Twenty-five carbon dioxide light traps were deployed in a treatment area and repeated in a control area. The treatment and control areas were reversed and sampled again the following season to allow for inter-area baseline and seasonal differences.

Nine water samples were taken and analysed for the presence of S-methoprene. Samples were taken from the outlet of chains of storm-water gullies during the first rainfall following application of S-methoprene briquets in the area.

Results

Storm-water gullies have been confirmed as a significant source of mosquito breeding and are likely to be increasing the spread of Ross River virus in the area in which they are located. Mosquito breeding peaks in early November, and decreases by February as the hot dry conditions prevent large scale breeding for all species.

Gullies produced a mean of 108 mosquitoes per day over the season. *Culex quinquefasciatus* and *Aedes notoscriptus* are the most abundant species within the Shire at all times in the season. Numbers of *C. quinquefasciatus* emerging can exceed 1600 per day per gully. *A. notoscriptus*

breeds to a lesser extent but can still exceed 70 adults per day. Both species are container breeders known to breed profusely in close contact with human habitation.

Treatment with S-methoprene is highly effective against both species for at least 70 days and partially effective for up to 120 days, treatment provided no control by day 124. A total of 90% control was given over the 124 days. Treatment has a significant impact on the abundance of *A. notoscriptus*, reducing the population by two thirds at the tail end of the season. This is likely to actively reduce the transmission of Ross River virus (RRV) within the treatment area.

Overall effectiveness of the briquet is not impacted significantly by the presence or absence of a float.

Treatment of storm-water gullies correlates with reduced abundance of *Aedes notoscriptus*, which is a competent vector of RRV, and was found to be an important transmitter of this disease, especially when numbers of *Culex annulirostris* are higher than usual.

Some improvements and supplements to the program are recommended, including timing of application and gully cleaning programs, and ongoing monitoring for priority vectors and evidence of pesticide resistance.

I certify that this thesis does not, to the best of my knowledge and belief:

- i. incorporate without acknowledgment any material previously submitted for a degree or diploma in any institution of higher education;*
- ii. contain any material previously published or written by another person except where due reference is made in the text of this thesis; or*
- iii. contain any defamatory material;*

Kerry Staples

11 April 2016

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CO₂ light traps were loaned from the Environmental Health Hazards, Medical Entomology unit within the Department of Health of Western Australia, and from the Shire of Mundaring, Town of Cambridge, and the Cities of Swan, Belmont and Melville.

Assistance with setting and retrieval of floating emergence traps and CO₂ light traps was provided by staff at the Shire of Kalamunda Environmental Health Service.

All environmental water samples were analysed by Analytical Reference Laboratory, Banksia Road, Welshpool, Western Australia.

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1. Introduction

Mosquitoes are a familiar pest to most people. Aside from ruining many a backyard barbecue, mosquito-borne diseases form the majority of the global burden of the vector borne diseases, which comprises 17% of the infectious disease burden (WHO, 2014). Mosquito control is a very important and effective public health intervention that has reduced the prevalence of Ross River virus in Australia (Tomerini, Dale and Sipe 2011). Within Southwest Western Australia, Ross River virus (RRV) and Barmah Forest virus (BFV) are the most commonly contracted mosquito-borne viruses. The absence of treatment or vaccines for the virus leaves vector control and personal mosquito bite prevention (including mosquito-proof housing) as the most important methods of preventing transmission (Russell 2002). Minimisation of nuisance biting and virus transmission prevention is the focus of local mosquito control programs.

This study closely examined the impacts of an anti-mosquito intervention of a single local authority in this region and included:

- Identification of local mosquito species and quantification of the productiveness of a key mosquito habitat;
- An examination of local disease transmission rates; and
- An evaluation of the effectiveness of the treatment method, including;
 - assessment of method of application on mosquito emergence rates;
 - evaluation of pesticide resistance;
 - quantification of impacts on absolute mosquito abundance in the area; and
 - evaluation of the potential environmental impacts of treatment.

By examining the linkages of these factors it has been possible to determine the efficacy of the existing program, and highlight areas of improvement to enhance protection of the local community from nuisance biting and disease carrying mosquitoes.

2. Literature Review

2.1. Endemic Species and Key Breeding Sites

The Shire of Kalamunda is a medium sized local government located in the east of the Perth Metropolitan Area. The study area consists predominantly of medium to large sized residential lots (500 to 4000m²). It has a Mediterranean climate of cool wet winters and hot dry summers with an annual rainfall of 725mm, Figure 2.1-1 (Bureau of Meteorology, 2015).

The study sites are the Foothills suburbs of High Wycombe, Maida Vale and Forrestfield, bounded by Kalamunda Road in the north, Dundas Road to the west, Hale/Hawtin Rd to the east and Tonkin Hwy to the south. Development in these areas occurred in two main waves in the 1970s and the early 2000's.

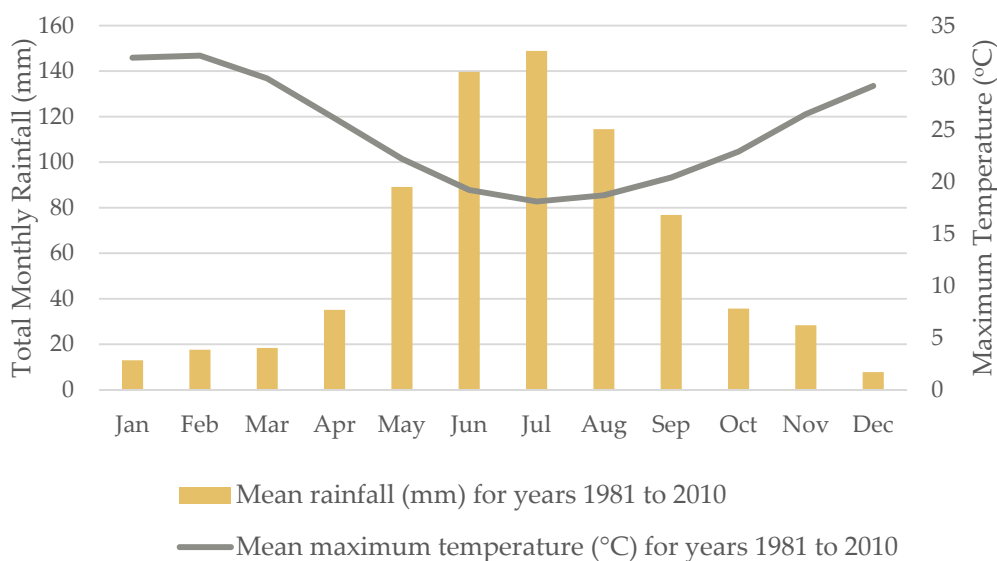


Figure 2.1-1 Mean daily maximum temperature and mean total monthly rainfall of study area.

The Shire does not have a significant number of natural mosquito breeding sites, such as swamps, lakes, or wetlands within its boundaries (Shire of Kalamunda – unpublished data), and overall has a lower level number of large-scale larval mosquito habitats, than areas with these features. This has been confirmed by the results of adult mosquito surveillance conducted by the Shire's Environmental Health Service annually since 2006 (Figure 2.1-2). These samples show the predominance of *Culex quinquefasciatus* and *Aedes notoscriptus*, both

peri-domestic breeders, occurring in manmade structures and items around the home. Also of note is the presence of *Culex annulirostris*, an important vector of multiple diseases (Hu et al., 2010), at 5% of the catch. An understanding of the defining characteristics of these species is necessary to effectively evaluate the design of the program.

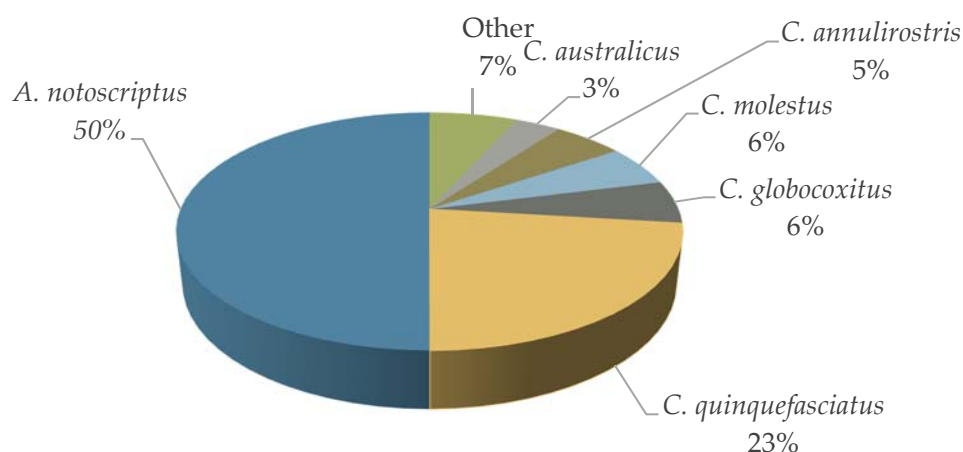


Figure 2.1-2 Proportions of Mosquito Species Collected in the Shire of Kalamunda 2006-2013 (Shire of Kalamunda – unpublished data).

Aedes notoscriptus

A. notoscriptus is the most common mosquito caught in the area comprising 50% of the total catch. A domestic and sylvan container breeder, *A. notoscriptus* will oviposit in a vast array of domestic sites containing fresh water, including; blocked rain gutters, pot plant saucers, ponds, tyres, tree and rock holes. It prefers sites with a rotting vegetation layer (Department of Health, 1991). It feeds on many vertebrates including humans, dogs, possums, and birds (Kay et al., 2007).

Larvae are quite adaptable, preferring temperatures between 18°C and 25°C, emerging at reduced numbers between 15 and 29°C (Williams and Rau, 2011), but failing to emerge at higher temperatures. Emergence ceases completely before temperatures reach 35°C (Williams and Rau, 2011). Daily estimated survivorship, or the proportion of adults surviving each day is between 0.77 and 0.79 (Watson, Saul & Kay, 2000), meaning a population of 100 adults would be reduced to less than 10 in 10 days.

Known as a vicious biter of humans, it feeds throughout the day, but prefers night feeding with peaks at dusk and dawn (Watson, 1998). It flies short distances, with a mean flight distance of around 130m (Verdonschot & Besse-Lotoskaya, 2014). Despite the short flight range Foley, Russell and Bryan (2004) found the species to be fairly homogenous, with Perth populations being closely genetically related to Eastern states populations.

Culex quinquefasciatus

C. quinquefasciatus has been the second most common collected species, comprising nearly a quarter of adults caught. Also a container breeder it utilizes has a wider variety of habitats as it will tolerate fresh and polluted waters. It does not travel far from larval habitats and swarms in the 30 minutes before sunset (Department of Health, 1991). Estimates of the average flight distance for this and similar species in the United States and Japan have had varying results, ranging from just 0.2km to 2.1km. Flight distance will vary between populations of the same species and also with differing terrain, habitat and other environmental factors (Ciota et al., 2012). Daily field survivorship for *C. quinquefasciatus* has been estimated at 0.871 to 0.883 (Elizondo-Quiroga et al., 2006), so it is more long lived than *A. notoscriptus*, taking 18 days for a population of 100 to be reduced to under 10.

Culex annulirostris

Culex annulirostris represents 5% of the number of adult mosquitoes caught in the Shire of Kalamunda, (Figure 2.1-2). Although a minor proportion of the average mosquito population it can breed in opportunistically produces large numbers of larva in ponded freshwater at temperatures above 25°C. Its numbers are likely to peak after rain events or flooding, and before ground pools dry up (Ritchie et al., 1997). It has been known to breed in storm-water drainage systems in other Australian states, and has a mean dispersal distance of 10km (Department of Health, 1991). This species takes blood meals predominantly on dogs, horses, possums, humans and birds (Kay et al., 2007), all of which are common within the study area.

Significant Breeding Sites

The available data show the most common species breeding in the study area are the container breeders, *C. quinquefasciatus* and *A. notoscriptus*. Both breed in close proximity to humans. They

are likely to breed in those permanent structures containing enough water to prevent them drying out over summer. The most common structures of this type in the study area are the sumps contained within the storm-water gully system.

Storm-water gullies are known to produce significant numbers of container breeding mosquitoes (Kay et.al, 2000). Storm-water gullies can produce up to 5 times the number of mosquitoes as a natural water source (Irwin, Hausbeck, & Paskewitz, 2008), and can extend the breeding season beyond its natural limits (Kwan et al., 2008). In similar habitats in the United States *Culex* species were found to be the predominant genus (92.4%), followed by *Culiseta*, *Aedes* and *Anopheles*, (Stockwell, P. et.al, 2006). *A. notoscriptus* has been found to breed in similar underground habitats in significant numbers in North Queensland (Kay et.al 2000).

Gullies are used to capture sand and to prevent pollutant runoff into natural waterways (Harbison, Metzger and Hu, 2010). There are two main entry styles; top entry, with a metal grate on top of the gully, or side entry, with a solid concrete cover, Figure 2.1-3. |

They all have inlet/outlet pipes either flush with the base, or higher than the base. Flush base types do not hold water for long periods unless there is a blockage downstream, and so they were not investigated in the study. Gullies with pipes higher than the base hold standing water

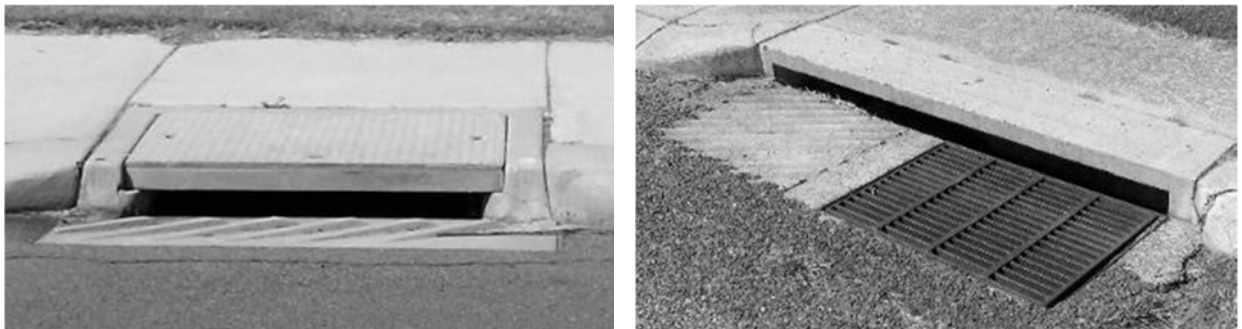


Figure 2.1-3 Side entry (left) and top entry (right) storm-water gullies

and provide mosquitoes with a suitable environment for oviposition and harborage (Knepper, LeClair, Strickler & Walker 1992). The interior of the gullies can be brick or precast concrete, round or square and of a range of dimensions. Gullies form chains that enter local compensating basins, ponds and creeks. There are approximately 5100 storm-water gullies throughout the Shire which are accessible from the surface and are generally located on the edge of paved and curbed streets. The median surface area of water in the gullies is 1.01m²

(Shire of Kalamunda - unpublished data). The medium to small residential areas that are subject to treatment with S-methoprene briquets in the Shire comprise approximately 6400ha. An average of 1600 wet gullies are treated annually giving an average of 0.25 wet gullies per hectare.

The existence of mosquitoes and their breeding sites is one factor that must be examined in assessing a mosquito control program. An understanding of their role in the transmission of disease within the area is also required.

2.2. Mosquito-Borne Disease

The predominant mosquito borne disease of human concern in this area is Ross River virus. Mean case attack rates for the past 5 years for suburbs within the Shire range from 0.37 to 1.59 per 1000 population, Table 2.2-1 (Jardine, A., Department of Health(WA), personal communication Nov 6, 2015). By comparison, the State mean attack rate for the same period is between 0.17 and 0.61 (Department of Health, 2015). Rates tend to be higher in the outer suburbs with closer proximity to natural animal virus reservoirs. Cases of RRV appear to be highest within the Shire between December and February each year (Table 2.2-1). Along with transmission from local fauna, an important mode of transmission may be via importation from other areas and then inter area transmission with humans acting as the reservoir (Russell, 2002). *Culex quinquefasciatus* has not been implicated as an important transmitter of RRV to humans, but both *A. notoscriptus* and *C. annulirostris* are considered to play very important roles in the RRV disease cycle with humans.

A. notoscriptus has been increasingly acknowledged as a very important vector of both Ross River and Barmah Forest viruses (Russell & Kay, 2004). It has been implicated in prolonging RRV epidemics and an increased number have been correlated with increased transmission of the virus in areas of inland Brisbane, especially when *Culex annulirostris* is also present (Hu, Mengersen, Dale & Tong, 2010). It has a high rate of infection with RRV during periods of virus activity (Ritchie et al., 1997).

Table 2.2-1 - Cases of Ross River virus, July 2010 to June 2015.

| Suburb | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Tot. | Pop. | Rate per 1000 |
|----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-------|------------------|
| Bickley | | 1 | | 1 | | | 1 | 1 | | 1 | | | 5 | 757 | 1.32 |
| Carmel | | | | | | | 1 | 1 | 1 | | | | 3 | 678 | 0.88 |
| Forrestfield | 2 | 1 | | | 1 | 2 | 3 | 8 | 3 | 2 | 3 | | 25 | 12965 | 0.39 |
| Gooseberry Hill | | 2 | | 1 | 1 | | 7 | 8 | 4 | 2 | 2 | | 27 | 3406 | 1.59 |
| High Wycombe | | | 1 | 1 | | 2 | 3 | 11 | 4 | | 1 | 1 | 24 | 12801 | 0.37 |
| Kalamunda | 2 | | | | 1 | | 5 | 6 | 7 | 3 | 1 | | 25 | 8077 | 0.62 |
| Lesmurdie | | | 1 | | | 4 | 7 | 2 | 5 | | 2 | 2 | 23 | 8203 | 0.56 |
| Maida Vale | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 5 | 4 | 1 | 1 | | 19 | 4138 | 0.92 |
| Pickering Brook | | | 1 | | | | | 2 | 1 | | | | 4 | 663 | 1.21 |
| Wattle Grove | | | 1 | | | 1 | 2 | 2 | 2 | 1 | | | 9 | 4422 | 0.41 |
| Walliston | | 1 | | | | 1 | | | 1 | | | | 3 | 1341 | 0.45 |
| Total (Shire) | 5 | 6 | 5 | 4 | 4 | 12 | 30 | 46 | 32 | 10 | 10 | 3 | 167 | 57451 | 0.58 |

A study conducted in Brisbane during a RRV epidemic found an infection rate in this species of 1.7 per 1000 mosquitoes (Ritchie et al., 1997). The presence of the virus in this species may provide an overwintering mechanism allowing the virus to be maintained in the area (Ritchie et al., 1997). Given its high affinity for the human environment it is likely to be a significant factor in human-mosquito-human transmission of RRV (Claflin & Webb, 2015).

C. annulirostris is a known vector of RRV and other arboviruses, especially in inland areas (Hu et al., 2010). It is acknowledged as the most significant vector of RRV in inland Australia (Hu et al., 2010), and had infection rates of 2.3 per 1000 mosquitoes during an RRV outbreak in Brisbane (Ritchie et al., 1997). Although not normally a large proportion of the mosquito

population within the Shire, *C. annulirostris* may introduce RRV into local hosts. *A. notoscriptus* may then distribute the virus more widely, making them jointly responsible for a significant amount of RRV transmission within the Shire. Along with an understanding of the local area, mosquito species and disease profile, an understanding of the effectiveness of the existing mosquito control program is needed.

2.3. Effectiveness of Mosquito Control Program

In Australia, it has been shown that long-term RRV incidence rates are lower in areas where active mosquito control programs are implemented (Tomerini, Dale and Sipe 2011). A good area-wide integrated mosquito management project aims to reduce the impact of the mosquito to within acceptable health and economic limits (Fonseca et al., 2012). To determine whether a program has been successful, it is necessary to be able to quantify a reduction in incidence of clinically presentation of disease and nuisance biting levels. The mandatory reporting of cases of RRV make it relatively straight forward to determine whether the number of clinical cases have declined, but measurement of the level of nuisance biting is more complex.

Nuisance biting can be broadly measured in two ways; counting complaints received by the responsible authority or by direct mosquito counts in the area. Complaints can be tracked over time; however, it is likely that the number of complaints received would be an underestimate of the experience of nuisance mosquito biting due to a reluctance to report or a lack of knowledge that such things can be reported. Additionally, when overall numbers of complaints are low statistics developed from those numbers can be heavily skewed by individual complaints and are unlikely to be a true reflection of the underlying mosquito problem. A direct count of the mosquito population is a more reliable estimate, but not without its own difficulties.

A nuisance biting rate has been defined in different ways. Carrieri et al. (2008) defined it as at 39 *Aedes* mosquitoes caught in a CO₂ trap per night. Informally, the Department of Health (WA) sets the level at 50 mosquitoes per CO₂ trap per night. Other definitions have been proposed, but these are the most relevant to this study. Both the Carrieri et al. (2008) method and the Department of Health (WA) method will be used in this study.

The programs most successful in achieving a reduction in mosquito-borne disease and nuisance biting have been those that included pre-emptive surveillance with extensive local knowledge

of mosquito habitats. A good mosquito control program should consist of monitoring mosquito species and abundance, public education programs, reduction or removal of breeding sites, and treatment of sources that cannot be modified (Fonseca et al, 2012). At the commencement of this study the Shire's program contained 3 of the 4 elements:

- Monitoring
 - Pre and post treatment adult sampling, using a CO₂ light trap, is conducted once in each of the seven most populated suburbs.
 - Mosquito complaints are investigated when received by the Shire's Environmental Health Officers and will include site inspection and advice or direction to rectify any breeding issues.
- Treatment
 - The only treatment method is applying a briquet containing S-methoprene to each storm-water gully holding water at the time of inspection. Inspection was done annually in December. The product label indicates briquets have a dry weight of 36g and contain 0.65g of active ingredient (18g/kg).
- Public education
 - Media releases at times of emerging mosquito-borne disease (as advised by DOHWA) and a flyer with personal mosquito control information available on the Shire website for the public to access.

The application of the briquets is one of the most important components of the program, yet the ability of the storm-water gullies in this area to support significant mosquito breeding, and the effectiveness of the chosen intervention for inhibiting breeding, has not been scientifically assessed. Studies of the effectiveness of S-methoprene in Western Australian storm-water gullies have not been conducted. Also of concern is the possibility of the development of pesticide resistance in the local mosquitoes and potential downstream impacts on native biota from pesticide application.

Storm-water gully breeding potential

Previous informal assessments of gullies with direct openings have shown that all were harbouring significant numbers of mosquitoes. Records from the annual mosquito control program indicate 33% of these of these gullies hold water at some point during the peak

summer months (range 18.1% to 47.12%) (Shire of Kalamunda – unpublished data). Data from North America has shown between 78 and 212 mosquitoes adult mosquitoes emerging from catch basins per day (Hamer, Kelly, Focks, Goldberg and Walker, 2011). Numbers of this scale are likely to make a significant contribution to overall mosquito abundance.

Treatment Effectiveness

The treatment used by the local authority is Prolink briquets. The active ingredient is S-methoprene. These are used because of their duration of activity.

S-methoprene is a juvenile hormone mimic which acts on larval stages and prevents metamorphosis. It is not effective on pupa (Wexler, 2005). The presence of S-methoprene does not deter oviposition by mosquitoes in the treated water, so will not drive mosquitoes to find other breeding sites (Butler, Suom, LeBrun, Ginsberg & Gettman, 2006). There are a number of formulations of S-methoprene available; liquid, sand, granule, pellet and briquet. Pellet formulations can be effective for up to 30 days (De Lauriers et al., 2006). The briquet formulation of S-methoprene is stated to be effective for up to 150 days, but is influenced by environmental factors such as time spent immersed under water, and sunlight exposure (Boxmeyer, Leach & Palchick et al., 1997).

The effective dose of s-methoprene varies between species and developmental stages. Half of third instar larvae of *A. notoscriptus* were killed (LD₅₀) at a concentration of 0.000359 ppm (Ritchie, Asnicar & Kay, 1997). Results for *C. quinquefasciatus* have been an LD₅₀ of between 0.005 and 0.0006 ppm for 4th instar larva and 0.0011 ppm for larvae of unstated instar (Navarro-Ortega, Marquetti, Valdes, & Garcia, 1991; Baruah & Das, 1996). The concentration preventing 50 % of pupal emergence for the same species was 0.0374 for treatment of 1st instar larvae and 0.00076 ppm for unstated instar larvae (Toma, Kamiyama, Fujihara, & Miyagi, 1990; Farghal, Roe, & Apperson, 1988).

Previous studies of S-methoprene briquets have shown that effectiveness decreases over the application period, but remains more effective than having no treatment (Stockwell et al., 2006). A United States study on the use of S-methoprene briquets found larval numbers were reduced by 69.5% over 17 weeks (Stockwell et al., 2006) in a warm wet summer environment. This study

was conducted in a hot dry summer climate, with much higher water temperatures, which may impact emergence rates of mosquitoes and the lifespan of the briquet.

The Prolink label (Appendix 1) requires the use of a float when applying briquets. This presents a significant burden of time and budget constraints, but is stated to be necessary to prevent coverage of the briquet by sediment which would inhibit dispersal of the active ingredient. This has been contested by the findings of studies on the effect of the presence of debris on S-methoprene efficacy. For instance, Baker and Yan (2010) found significantly lower mosquito emergence rates from gullies with sediment versus clean gullies in a Canadian trial; 1% vs 2% just after application, 4% vs 16.5% at day 54 and 12% vs. 45% after day 96 (Baker & Yan 2010). S-methoprene adsorption onto organic compounds may be responsible for this effect (Baker & Yan, 2010). Again, differing climate and other environmental factors do not mean this result would be applicable to Southwest Western Australia.

Pesticide Resistance

The local authority has been using S-methoprene annually for 15 years. Continuous use of the same product can lead to resistance, and this has been reported in a variety of species and locations:

- High resistance to liquid S-methoprene has developed in *Aedes nigromaculis* (Ludlow) in California following 20 years of use (Cornel, Stanich, McAbee & Mulligan, 2002);
- Resistance was induced in *Culex quinquefasciatus* by selection in laboratory rearing (Amin, 1984 in Cornel et al., 2002);
- Suspected resisted to S-methoprene has been documented in wild *Aedes albopictus* in Florida, United States (Marcombe et al., 2014).

Resistance in Western Australia has not yet been reported, but given that it is in common use, monitoring for development of resistance should be undertaken.

Environmental Impacts

Application of S-methoprene has been encouraged to protect of public health; however, there is an increasing level of community concern about the use of any chemical or pesticide by local and state authorities, and an increasing expectation that any possible impacts on the

environment have been assessed. S-methoprene can have negative impacts on other insect larvae, amphibians, crustaceans and fish (Kuo et al., 2010). Local area studies are required as the effectiveness of S-methoprene is influenced by local environmental factors such as; sediment components, water volume and depth, temperature, exposure to ultraviolet light, microbial degradation, frequency of gully cleaning programs, and target species (Baker and Yan, 2011; Butler, Lebrun, Ginsberg & Gettman, 2006). S-methoprene is not released from briquets at a consistent rate, so multiple samples should be taken. Sampling directly for its presence can also be difficult. Rainfall can cause re-suspension of S-methoprene, and concentrations are higher in upper layers unless mixing occurs (Des Lauriers et al., 2006). Sampling for effects of non-target organisms may be a useful indicator, but is outside of the scope of this study.

Post treatment environmental S-methoprene has been detected at levels ranging from 0.4 to 0.14µg/L in a North American study of storm-water gullies using briquets (Butler, Lebrun, Ginsberg, & Gettman, 2006). This is below levels deemed to negatively impact non-target species; however, actual levels may have been underestimated due to problems with the sampling protocol (Kuo, McPherson, Soon, Pasternak & Garrett, 2010). Another study in Canada found S-methoprene levels exiting the storm-water system using 0.7 g of 4.25% S-methoprene pellets did not exceed recommended limits however not all rain events were sampled in the study (Des Lauriers et al., 2006).

There has been no maximal acceptable level set for S-methoprene in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (Australian and New Zealand Environment and Conservation Council, 2000) or the Guidelines for Managing Risks in Recreational Water (National Health and Medical Research Council, 2008). However, a level has been set for Canada of 0.9 µg/L (Canadian Council for Ministers of the Environment, 2007).

3. Methodology

3.1. Emergence Trapping

Laboratory rearing of adults from field-collected larvae is not considered suitable for estimating population numbers as environmental conditions and other factors that impact on the proportion successfully emerging are not assessable in an artificial setting (Hamer et al., 2011). Floating emergence traps (Figure 3.3-1 and 3.1-2) located in storm-water gullies are a preferred option as they will only catch newly emerging adults. Ovipositing females or resting adults will not be trapped. Pupal avoidance or preference for the trap is considered negligible (Hamer et al., 2011). To determine the number of mosquitoes emerging from the storm-water gullies in this study both field sampling and laboratory simulations were conducted.



Figure 3.1-1 Set of 3 pyramidal emergence traps placed in a storm-water gully.



Figure 3.1-2 Diagram of emergence collection jar which is fixed to the top of the emergence trap for trapping of newly emerged adults.

Gullies in High Wycombe, Maida Vale and Forrestfield that contained water during the previous mosquito control program were selected for field sampling. Gullies were inspected for the presence of water of sufficient depth (>5cm) as these were considered most suitable for ovipositing females (Knepper et al., 1992). Thirty gullies that met the inclusion criteria were allocated a number. A random number generator was then used to assign 15 gullies to the control group, or one of two treatment groups (S-methoprene briquet that were allowed to sink or to float) for sampling. To reduce cross-contamination of S-methoprene during rainfall, treated gullies were located down-stream of controls where they are identified as being on the

same drainage line (Butler et al., 2006). Gullies are not subject to cross contamination from treatments outside of the sample area as the drainage lines do not connect. Emergence trapping was conducted using square pyramidal emergence traps based on the design of Walton (1999), but with a fixed leg, non-collapsible, stackable design. The surface area covered was 0.025m². The collection jar was created from two clear plastic containers with the bases removed and glued together with a clear water-proof silicone. A white plastic kitchen funnel was cut to fit the opening of the jar as shown in Figure 2.1-2. The opening of the top jar was covered with fine white fabric mesh secured with an elastic band. A lid from a jar had a hole cut into it leaving the rim intact and was glued to the top of the pyramidal trap. The collection jar could then be easily screwed to the top of the trap and removed at collection for compact transport. The lid from the second jar was used to cover the bottom opening of the collection jar when unscrewed from the trap.

Three traps were placed in each gully to allow for uneven larval densities across the surface of the water (Figure 2.1-1). Traps were placed once per month and collected after seven days in the field to minimize catch degradation (Hamer et al., 2011). Collection jars were frozen for a minimum of 30 minutes, then adult mosquitoes were extracted from the jar using tweezers and identified to species level using morphological keys. Mean daily breeding per square meter of gully area was calculated by dividing by; the area of trap (0.0625m²), expected proportion (0.36), and number of days the trap was set (n=7).

Two distinct rounds of sampling were conducted. Round 1 was conducted in March and April 2014. Treatment had been applied one week prior to the March sample. Round 2 was conducted from October 2014 to March 2015. Treatment was applied immediately after collection of the November sample.

Groups were as follows:

- Control - 5 gullies with no treatment;
- sink - 5 gullies with 1 Prolink XR briquet and no float; and
- sink - 5 gullies with 1 Prolink XR briquet attached to a float contained in a fiberglass mesh bag attached to a plastic foam float with a cable tie. Floats were attached to the

gully entry with fine rope to prevent them being swept down drainage lines during rainfall.

The mesh bag allowed free flow of water so should not impede the flow of S-methoprene out of the bag. At the time of trap placement and again at retrieval the following details were recorded: water temperature, depth of water, depth of sediment, density estimate for 1st, 2nd, 3rd and 4th instar larva and pupa. Temperatures were measured using a calibrated probe thermometer immersed in the surface water for one minute. Depth of water and sediment was measured using a pole with 10mm increments. Density estimates for larva were obtained using a larval scoop. Three scoops were taken from each gully from different sections of the surface, and an average density obtained. Low was from 1 to 5 larval per scoop, medium was from 10 to 20 per scoop, high was 20 or more per scoop. Data on gully dimensions and surrounding development; paved, grassed, open or shaded was also collected. The floats were examined to ensure they still contained a briquet. It was not possible to locate briquets without a float due to high turbidity of the water. Air temperature and rainfall data was obtained from the Perth Airport weather station at www.bom.gov.au).

Laboratory study

To determine what proportion of emerging adults were collected in the emergence jar six simulations were conducted. Pupa and the water from the gully were collected. Pupa were kept in the gully water to increase survivorship (Williams and Rau, 2011). Emergence traps were placed in large plastic containers and then into cardboard boxes cut to simulate gully light conditions, 3 top entry and 3 side entry designs. Trials were kept at ambient outdoor shade temperatures. Trials were run for 7 days, then adult mosquito samples were placed in a freezer for 30 minutes to kill them. They were then identified to species level.

3.2. Adult Sampling

To determine species and abundances of female adult mosquitoes in the area 25 CO₂ light traps were used at approximately 100m intervals in a grid pattern. Sampling was conducted for five consecutive nights in each location in 2014 and 4 consecutive nights in 2015, refer to timeline, section 4.1). To determine if there was a correlation between treatment of gullies and adult female population treatment and control status was reversed for year 2. This method best assesses impacts on species with smaller flight distances, such as *A. notoscriptus*. Apparent

impacts on local breeding of species with longer flight distances, such as *C. quinquefasciatus* will be more difficult to determine due to influx from untreated surrounding areas. The location of CO₂ traps and gullies containing water at the commencement of CO₂ trapping are shown in Figures 3.2-1 and 3.2-2.



Figure 3.2-1 - Location of CO₂ traps and gullies containing water - Maida Vale. Red dots indicate trap locations. Blue dots indicate gullies containing water.

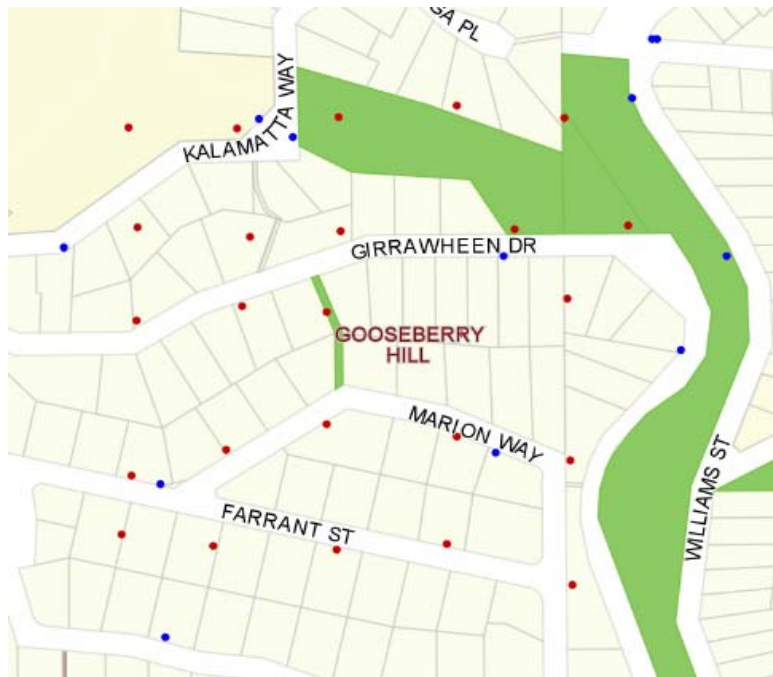


Figure 3.2-2 - Locations of CO₂ traps and gullies containing water - Gooseberry Hill. Red dots indicate traps, blue indicated a gully containing water.

3.3. Mark Release Recapture

To estimate dispersal distances a mark-release-recapture study was conducted. A total of 531 adult mosquitoes were used in a mark and release study. Altogether, 337 were reared in the lab from wild caught larva and pupa. These adults were fed using a 10% sugar syrup (Russell, Webb, Williams & Ritchie, 2005). 194 adults were collected via CO₂ light trap the night prior to release. A subsample of 40 (7.53%), a mixture from both groups, were retained for a survival analysis.

The remaining 491 mosquitoes were stunned by exposure to dry ice for 30 seconds then marked with pink dust (CO₂ caught) or orange dust (reared) using a small bulb duster (Dickens and Brant, 2014), Figure 3.2-3. They were released from the center of the control site in March 2014, day 0. Traps were set the evenings of day 1, 2, 3, 4 and 5 between 3pm and 5pm and retrieved the following morning between 8am and 10am. Samples were checked for the presence of marked mosquitoes using an ultraviolet light.

Survival Analysis

Of the retained sample, the 40 adults were divided into 3 groups, the control group (n=17), group exposed to dry ice (n=12) and group exposed to dry ice and dusted (n=11). Groups were kept separate. Dead mosquitoes were removed and identified daily.

3.4. Environmental Water Sampling

Samples from gully discharge points, Figure 3.4-1) where the date of upstream gully treatment is known were taken when gully chain outflows began to flow following significant rainfall (Kuo et al., 2010). Samples were stored in an esky with an ice brick to maintain temperatures $<5^{\circ}\text{C}$ and transported immediately to an analytical laboratory for analysis.

Acid treated polycarbonate containers were provided by the analytical laboratory for sample collection. Containers were used only once to avoid adsorption of S-methoprene onto surfaces. Prior to analysis laboratory glassware was treated to minimise active adsorption sites. Samples were analysed for residual S-methoprene.



Figure 3.4-1 Environmental sampling point - outflow discharge pipe of a chain of storm-water gullies in the Shire of Kalamunda



Figure 3.2-3 Marked mosquitoes being transported to release site. Mosquitoes were fed a 10% sugar solution via a soaked cotton wool ball.

4. Results & Observations

4.1. Statistical Analysis and Timeline

Mosquito catch results from Floating Emergence Traps (FET's) and CO₂ light traps are count data and do not follow a normal distribution (Figures 4.2-1, 4.2-3, 4.3-2, 4.3-3). Sample size for each area is small (17 FET's and 25 CO₂ traps in each area), so a normal approximation was not appropriate. Unless otherwise specified data was analysed in SPSS using Generalised Estimating Equations (GEE) for repeated measures designs. A negative binomial model was used as the data is over-dispersed with the variance much larger than the mean. A robust estimator was used with an independent correlation matrix as this gave the lowest QIC (Quasi Likelihood under Independence Model Criterion) value (Garson, 2013).

A timeline of treatments and sampling is as follows:

- January 2014 – treatment of gullies in Gooseberry Hill
- 4th to 8th March – CO₂ light trapping in control area
- 18th March – marked mosquitoes released in treatment area
- 19th to 23rd March - CO₂ light trapping in treatment area
- 28th March to 7th April – FET's in field
- 25th April to 2nd May – FET's in field
- 9th to 16th October – FET's in field
- 6th to 13th November – FET's in field
- 13th November – treatment of gullies
- 10th to 17th December – FET's in field
- 14th to 21st January 2015 – FET's in field
- 11th to 18th February – FET's in field
- 4th to 7th March - CO₂ light trapping in control area
- 10th to 13th March - CO₂ light trapping in treatment area
- 11th to 18th March – FET's in field

4.2. Emergence Trapping

Laboratory Study

Six gully simulations were run. The mean proportion of larva surviving to emergence as adults and being caught in the emergence jar was 0.3592. The proportions were roughly normally distributed (Figure 4.2-1). This proportion, 0.36, (95% C.I. 0.024 to 0.48) was significantly different to the Hamer et al. (2011) value of 0.5 (1 sample t-test, $p=0.028$, 5df).

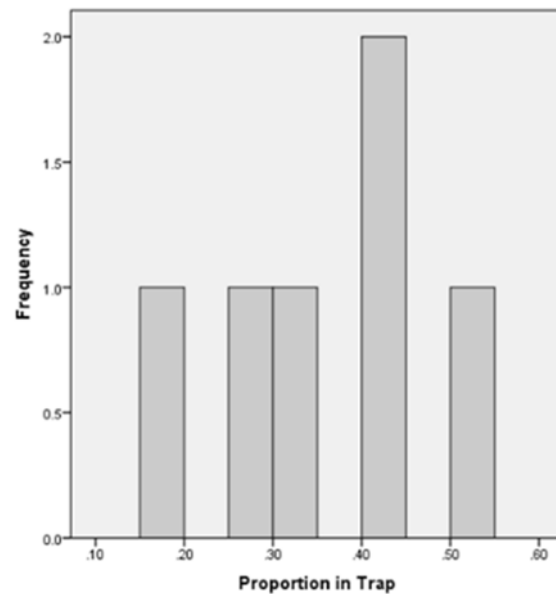


Figure 4.2-1 Proportion of emerging adults caught in emergence jar

The number of adults caught in each style of gully was not equal. Over 1.6 times more adults were caught in a top entry gully (44.6%) than a side entry gully (27.3%), tested by independent t-test ($p=0.038$, equal variances not assumed). The mean difference in proportion was 0.173 (95% C.I. = 0.01545 to 0.3306). However, sample size was small ($n=6$) so results should be used with caution therefore the mean proportion of all trials will be used (0.36). Using the overall mean in this way may result in an overestimate or underestimate of the true number of mosquitoes emerging, however the type of gullies in the field was mixed (5 top and 10 side), therefore at the scale of the whole area the difference should be minimal, and would not be of an order of magnitude.

Field Results

Numerous ($n=289$) successful emergence trapping events were conducted, 71 for sink treatment, 73 for float treatment and 145 controls. A successful event was defined as one during which the float remained intact and with all bottom edges of the trap in contact with the water surface at retrieval. 28 were unsuccessful (8.83%). A total of 4,511 adult mosquitoes were caught over the period, of which 2,281 were female (50.6%) and 2,230 male (49.4%). *C. quinquefasciatus* was the most common species (97.3%), with *A. notoscriptus* the next most common (2.3%). Other species caught in negligible numbers included; *Culex australicus* ($n=12$), *Aedes alboannulatus* ($n=2$) and *Coq. nr. linnaeus* ($n=1$). Once adjusted to numbers per metre square of gully water per

day, ranges of emergence were up to 1,625 *C. quinquefasciatus* per day per meter square in November. This is an underestimate as samples from this period were wet and some become encased in mud and were unable to be sufficiently identified. Peak emergence of *A. notoscriptus* was in December, with the maximum emergence rate being 74 per day. There was a correlation between the presence of 1st/2nd instar and 3rd/4th instar larvae and pupa and the number of adults emerging from the traps (Sig = 0.011, 0.016 and 0.000, respectively), however absence of larva was not indicative of a nil count at trap retrieval.

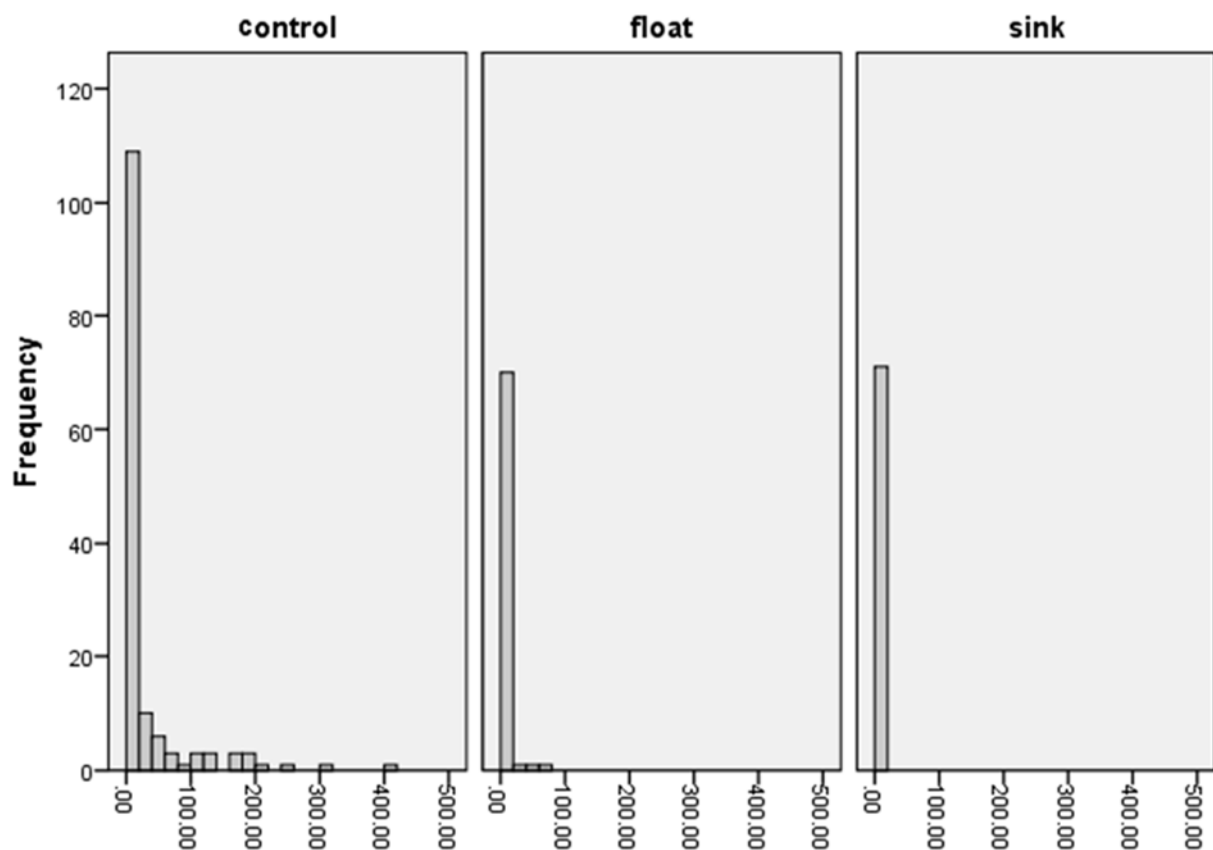


Figure 4.2-2 Distribution of count values for *C. quinquefasciatus* by treatment type.

Effectiveness of Sink and Float Treatments

Distribution of count values for control, sink and float treatments for *C. quinquefasciatus* and *A. notoscriptus* are shown in Figure 4.2-2 and 4.2-3. Both figures show a reduction in the maximum number of mosquito counts for the float and sink treatments when compared to control. Over the trial period sink treated gullies (n=71) showed a lower marginal mean than float (n=73)

(unadjusted number of adults per trap = 1.39 vs 3.13, respectively), however treatment type was not a significant factor ($p=0.329$, QICC = 230.846).

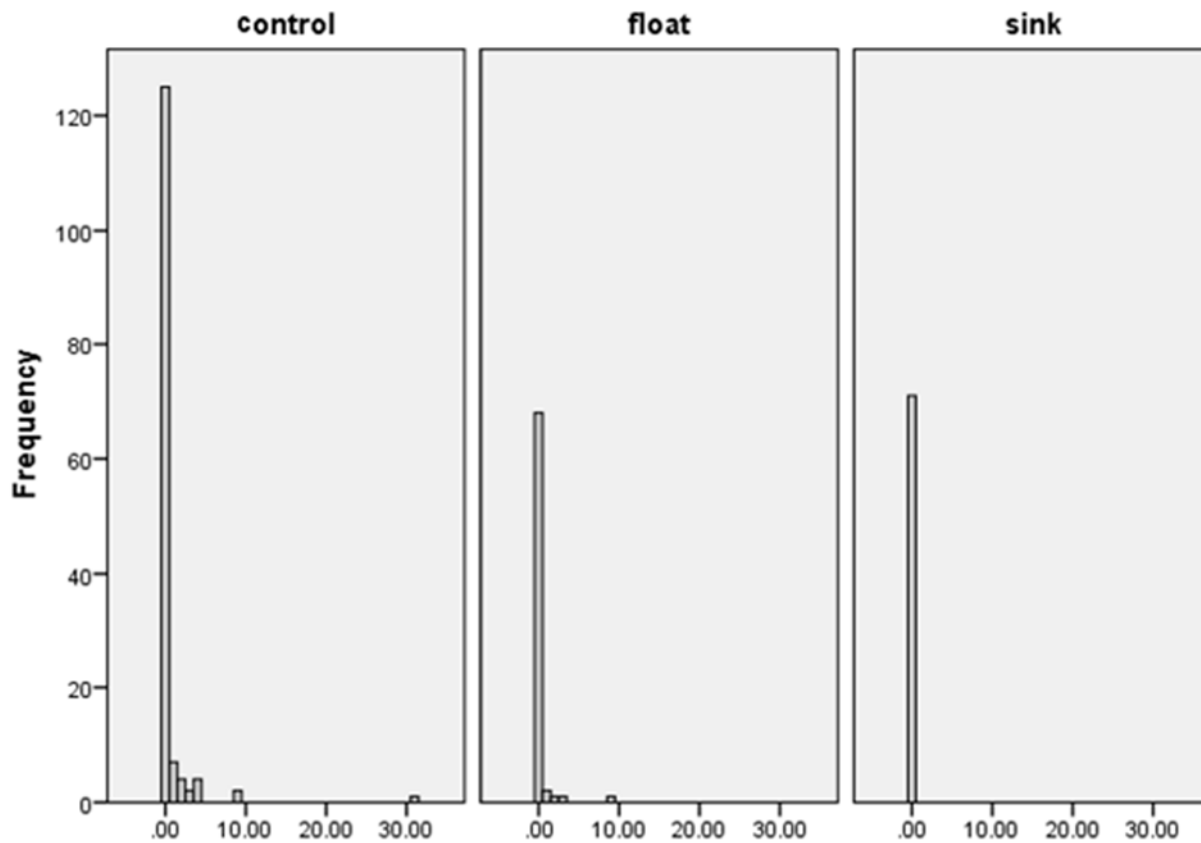


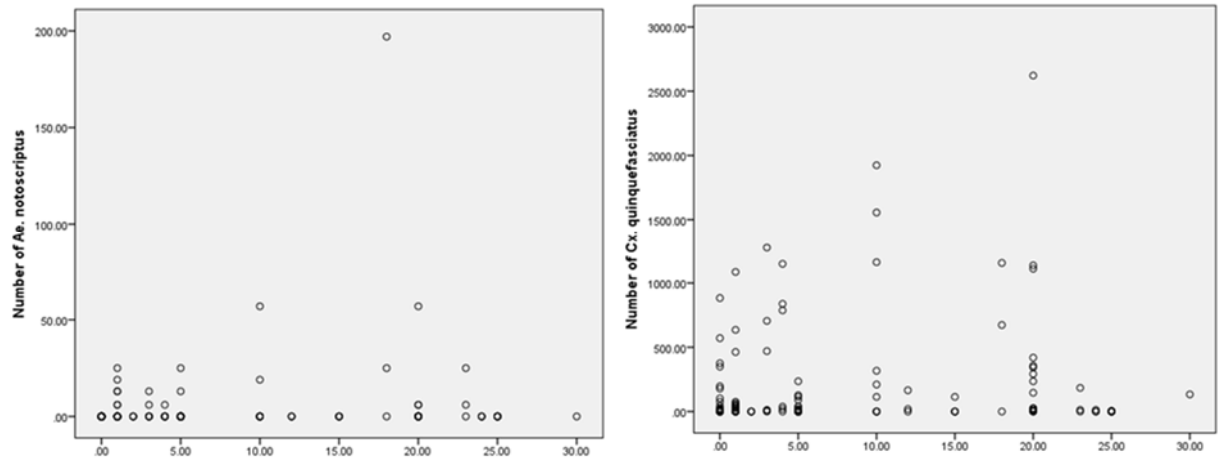
Figure 4.2-3 Distribution of count values for *A. notoscriptus* by treatment type

Environmental Factors – Temperature, Depth and Sediment

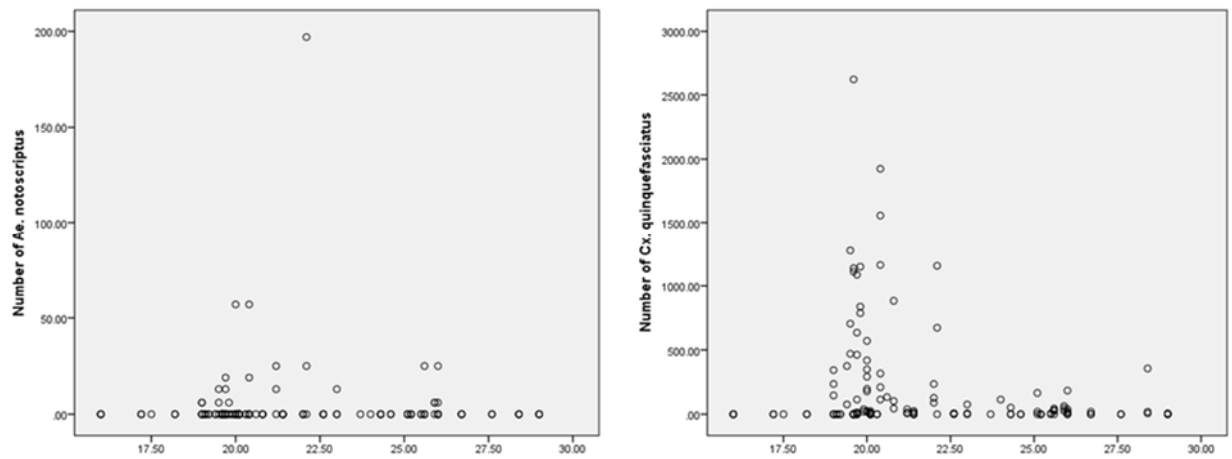
Figure 4.2-4 shows the relationship of environmental factors against total number of adults caught in the FET's by species. Recorded water temperatures had a mean of 22.6°C and ranged from 17.9°C to 28.7°C, mean water depth was 266mm and ranged from 0 to 630mm. Mean sediment depth was 69mm and ranged from 0 to 400mm. Peak breeding for water depth was near 200mm with reduced breeding at either extreme. Breeding numbers start to increase at above 18°C, peak at between 20 to 22°C and is positively skewed, tapering off between 28 and 30°C, although this was less marked for *A. notoscriptus*. The distribution of breeding against depth of sediment is more variable across the range of depth. Water temperature, water depth and sediment depth were recoded into ordinal variables to use as a factor in the GEE model.

Temperature

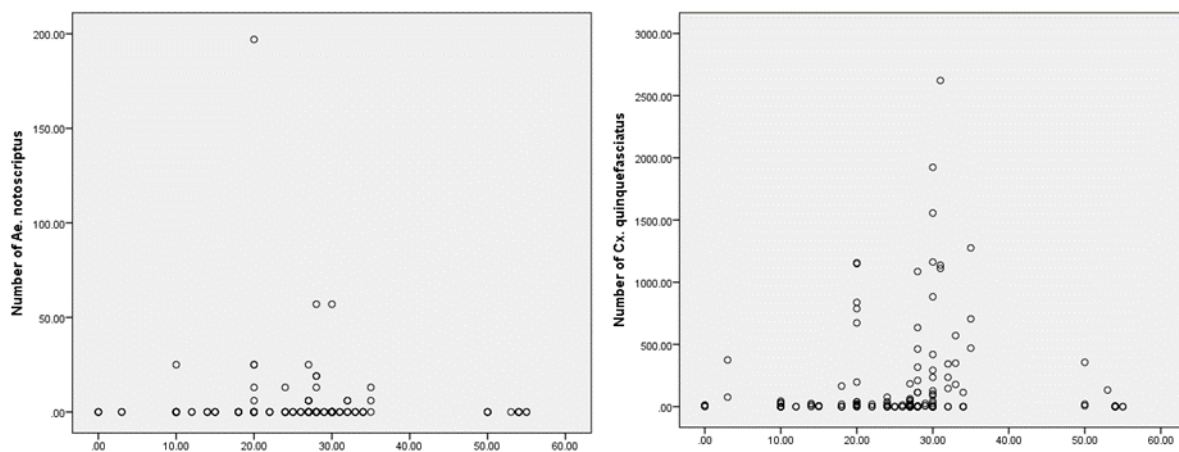
Water temperature (n= 145, QICC 587.511) was a significant predictor of mosquito emergence (p= 0.000, 4df). Breeding was significantly lower at temperatures above 24 °C. Figure 4.2-5 shows the estimated marginal means. *A. notoscriptus* bred at ranges from 17.9 to 25.8°C. *Culex quinquefasciatus* bred between 17.9 and 28.7°C.



(a) – Depth of Sediment (cm)



(b) – Water Temperature (°C)



(c) – Water Depth (cm)

Figure 4.2-4 Scatterplots of depth of sediment, water temperature and water depth vs number of adults mosquitoes (*A. notoscriptus*, left, and *C. quinquefasciatus*, right) caught in FET in untreated gullies. Dark coloured circles indicate more than one sample

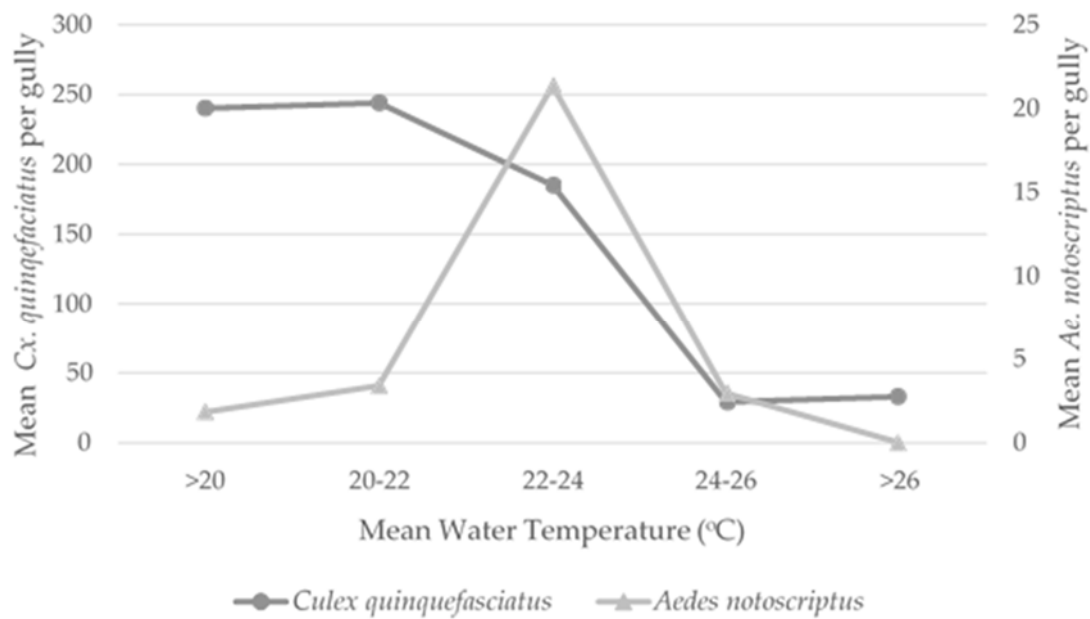


Figure 4.2-5 Mean adult emergence by temperature level and species.

Treatment Effectiveness

For the study period overall, when compared to control, treatment type was a significant predictor of count values for the gullies ($p=0.000$, QICC = 711.703). A best model fit was obtained using gully temperature, depth of water and depth of sediment as factors in the analysis. Untreated gullies produced over six times more adults than treated gullies. The unadjusted mean number of adults for control gullies was 17.1 (95%CI 11.0-26.7), 2.7 (95%CI 1.3-5.9) for float and 0.7 (95%CI 0.4-1.3) per day for sink treated gullies. Adjusted values were 108 mosquitoes per day for control gullies, and 17 and 4 per day for float and sink, respectively. Mean adjusted breeding by treatment type is shown against water temperature, relative humidity and mean maximum air temperature for the month of trapping in Figure 4.2-6. The pattern of gully water temperature follows air temperature closely. Relative humidity dips below 40% after November and remains so for the rest of the season. Analysis using the same model was applied to results by month. Table 4.2-1 shows the results of analysis within each month. Significant results were shown for all samples within 69 days of treatment.

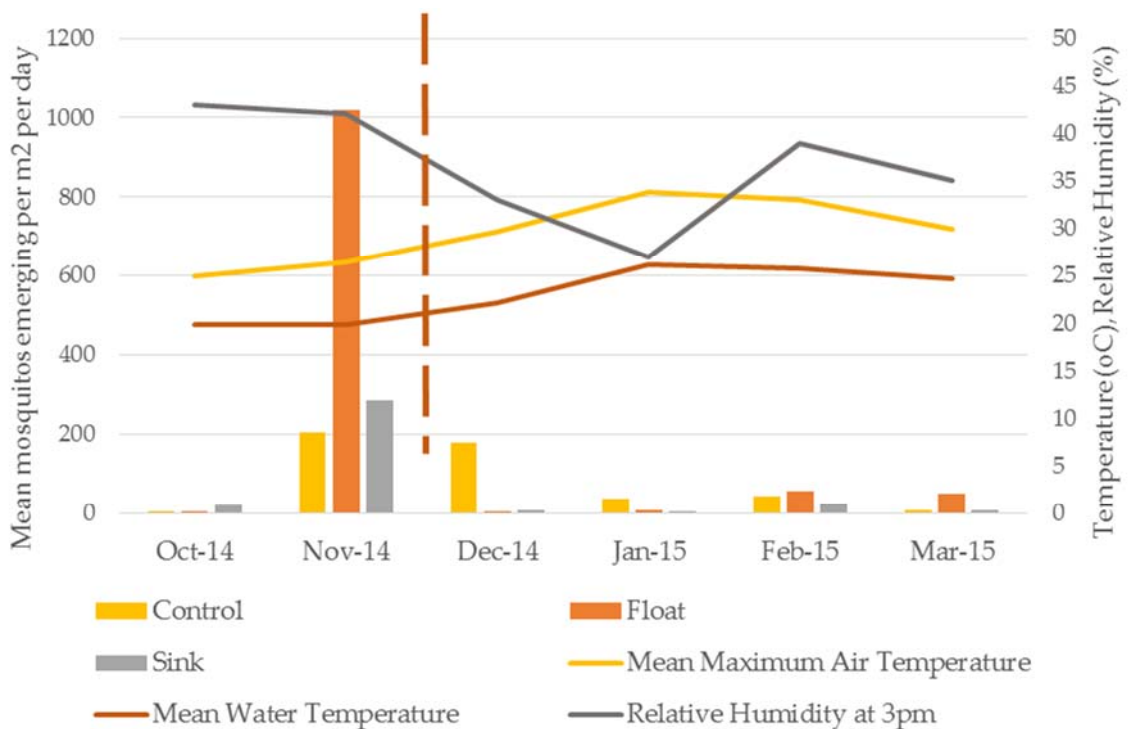


Figure 4.2-6 - Mean number of adults emerging per day per m^2 , mean air temperature, relative humidity and gully water temperature by month and treatment type. Dashed line indicates time of treatment with *S-methoprene*.

To determine if there was any pre-existing correlation between amount of breeding within groups. October and November 2014 were analysed using the allocated treatment categories. Results for November indicate a significant relationship, and show that compared to control, the float has a significantly higher marginal mean than the control ($p=0.000$). For December and January there was a significant decrease compared to control for float and sink treatments ($p=0.000$). There was no significant difference between groups in February. It was observed that the Prolink briquets in the float treated gullies had completely dissolved by this time (96 days post treatment). It was not possible to observe the size of briquet in the sink treated gullies could not be observed due to excessive turbidity of gully water and sediment coverage. By March there was a significant difference with breeding in float treated gullies again exceeding sink and control gullies.

To further quantify the effectiveness of the treatment, Mulla's Formula and assumptions of independence of samples, a fixed ratio of productivity between gullies (i.e. highly productive gullies are always so and vice versa), and that any alteration in the relative productivity is the result of the treatment (Reisen, 2009) was used to give the Estimated Percent Control and allow for abundance fluctuations due to seasonal variation.

Mulla's Formula:

$$\%R = 100 - \left[\frac{c_1}{t_1} * \frac{t_2}{c_2} * 100 \right]$$

Where:

c_1 = control gullies pre-treatment mean;

c_2 = control gullies post-treatment mean;

t_1 = treatment gullies pre-treatment mean;

t_2 = treatment gullies post-treatment mean.

This formula was also used by Knepper et al. (1992) for analysis of briquet performance in storm-water gullies. Figure 4.2-7 shows the results as a percentage of control provided, by month from November 2014 (pre-treatment) to March 2015 (final post-treatment sample). Treatment was above 95% for the first 70 days. Both treatments continued to control up to two thirds of breeding for 3 months (96 days), however by the end of the study, at 4 months or 124 days, negligible control was provided. Cumulative mean emergence from December to March, with cumulative percent control adjusted using Mulla's Formula, is shown in Figure 4.2-8. Overall float and sink controlled gullies produced 10.1 and 8.1 percent of the untreated gullies, respectively.

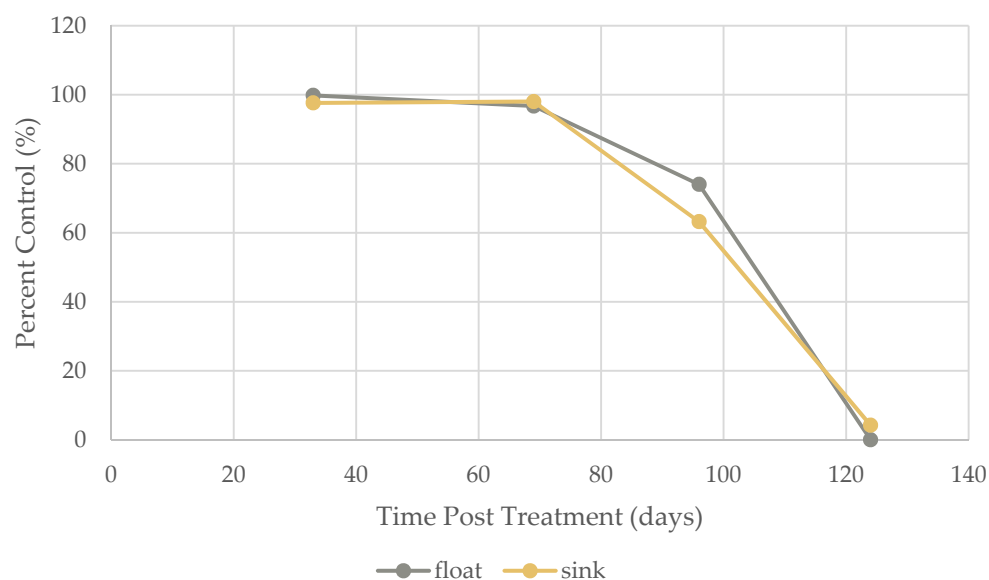


Figure 4.2-7 – Percent control, of mosquito emergence in gullies treated with a Prolink briquet.

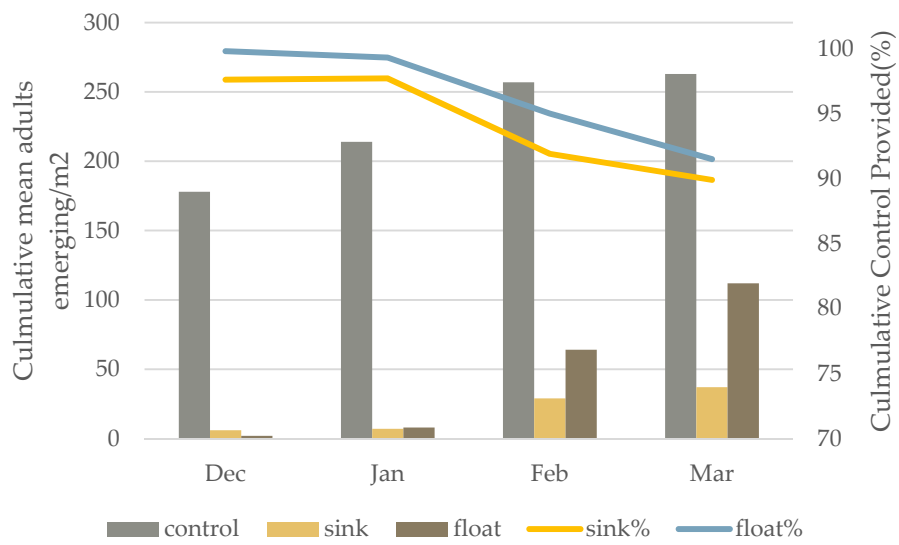


Figure 4.2-8 - Cumulative mean emerging mosquitos and cumulative percent control by month and treatment type.

Table 4.2-1 - Results of GEE analysis by month

| No. samples | Days since treatment | QICC | p-value | Adjusted Marginal Means (per m ² per day) | | |
|----------------|----------------------------|------|---------|---|-------|------|
| | | | | mean, upper 95%CI, lower 95%CI | | |
| | | | | control | float | sink |

Pre Treatment

| | | | | | | | |
|---------------|----|---|--------|-------|-----|------|-----|
| Oct-14 | 32 | - | 64.151 | 0.145 | 3 | 3 | 20 |
| | | | | | 1 | 1 | 3 |
| | | | | | 7 | 18 | 122 |
| | | | | | 204 | 1020 | 284 |
| Nov-14 | 38 | - | 79.314 | 0 | 112 | 671 | 95 |
| | | | | | 372 | 1551 | 846 |

Post Treatment

| | | | | | | | |
|---------------|----|-----|---------|-------|-----|-----|----|
| Dec-14 | 43 | 33 | 92.639 | 0 | 178 | 2 | 6 |
| | | | | | 41 | 1 | 2 |
| | | | | | 773 | 5 | 18 |
| | | | | | 36 | 6 | 1 |
| Jan-15 | 43 | 69 | 59.122 | 0 | 18 | 2 | 0 |
| | | | | | 72 | 24 | 3 |
| | | | | | 43 | 56 | 22 |
| Feb-15 | 41 | 96 | 135.732 | 0.605 | 16 | 13 | 5 |
| | | | | | 113 | 249 | 86 |
| | | | | | 6 | 48 | 8 |
| Mar-15 | 25 | 124 | 46.755 | 0.001 | 2 | 27 | 2 |
| | | | | | 22 | 83 | 28 |
| | | | | | 89 | 11 | 25 |
| Mar-14 | 25 | 14 | 37.296 | 0 | 35 | 7 | 25 |
| | | | | | 227 | 15 | 25 |

4.3. Adult Sampling

Traps were set at 25 locations in the treatment and control areas. Areas were selected by availability. These areas had not been subjected to treatment under the Shire's annual program at the time of the study. Overall 404 successful trapping nights were conducted, of which 204 were in the treatment area and 200 in the control area (221 in Gooseberry Hill and 183 in Maida Vale), Figures 3.2.1 and 3.2.2. A successful trapping night was defined as one in which the fan was still operational at the time of retrieval, and the catch bag was still intact and in place. 5287 adult mosquitoes were caught in total. 11 different species were present, Table 4.3-1.

Table 4.3-1 - Total number of mosquitoes caught by CO₂ trap by species.

| Species | Total | Percent |
|-------------------------------|-------|---------|
| <i>Culex quinquefasciatus</i> | 3289 | (62.2%) |
| <i>Aedes. notoscriptus</i> | 1649 | (31.2%) |
| <i>Culex australicus</i> | 90 | (1.7%) |
| <i>Culex annulirostris</i> | 76 | (1.4%) |
| <i>Anopheles annulipes</i> | 45 | (0.9%) |
| <i>Coq. nr. linnaeus</i> | 43 | (0.8%) |
| <i>Culex globocoxitus</i> | 37 | (0.7%) |
| <i>Tripteroides atripes</i> | 6 | (0.1%) |
| <i>Aedes hesperontius</i> | 4 | (0.1%) |
| <i>Culiseta atra</i> | 3 | 0.1% |
| <i>Aedes sagax</i> | 1 | <0.1% |

Figure 4.3-1 shows the distribution of the two main species by treatment type and area. An unadjusted mean of 7.37 (control) and 8.90 (treatment) for *C. quinquefasciatus* and 1.64 (treatment) and 6.57 (control) for *A. notoscriptus* were caught in the traps each night.

Trapping was conducted in March. The results of the emergence trapping would indicate that there is less mosquito activity at this time.

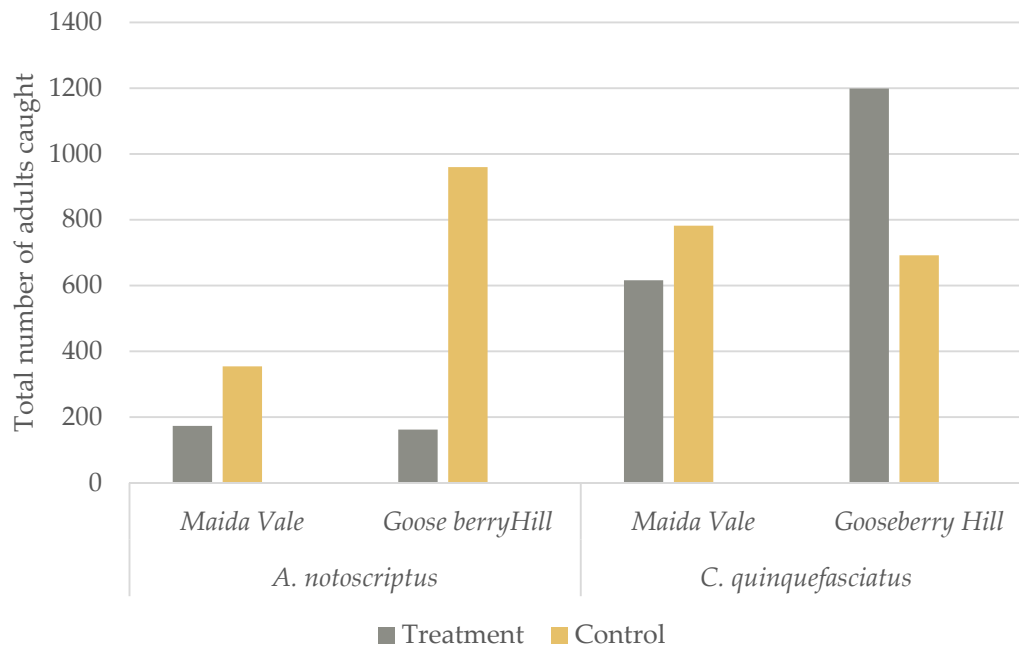


Figure 4.3-1 Total adults caught by species, treatment and area.

The number of gullies containing water during the CO₂ trapping was recorded. There were 23 in the Maida Vale area and 12 in the Gooseberry Hill area, Figures 3.2-1 and 3.2-2. This gives wet drain densities of approximately 1 per hectare and 0.5 per hectare respectively. The density of *Culex* sp being released from storm-water gullies was determined using the mean number of wet gullies per hectare and the mean daily emergence as determined by Floating Emergence Trapping. The mean daily density was 91 and 183 *Culex* per hectare in Gooseberry Hill and Maida Vale, respectively.

A Voroni diagram was used to determine whether there was a correlation between the number of wet drains and the number of mosquitoes caught at the corresponding trap. The diagram was constructed using trap locations as seeds. A polygon section is constructed around each seed so that all points within the section are closer to that seed than any adjoining seed. The number of wet drains located in each Voroni segment was used in the GEE analysis model.

Histograms of the main species caught are shown in Figures 4.3-2 and 4.3-3. The distributions of *A. notoscriptus* shows an increase in the number of zero counts and a decrease in the maximum count values.

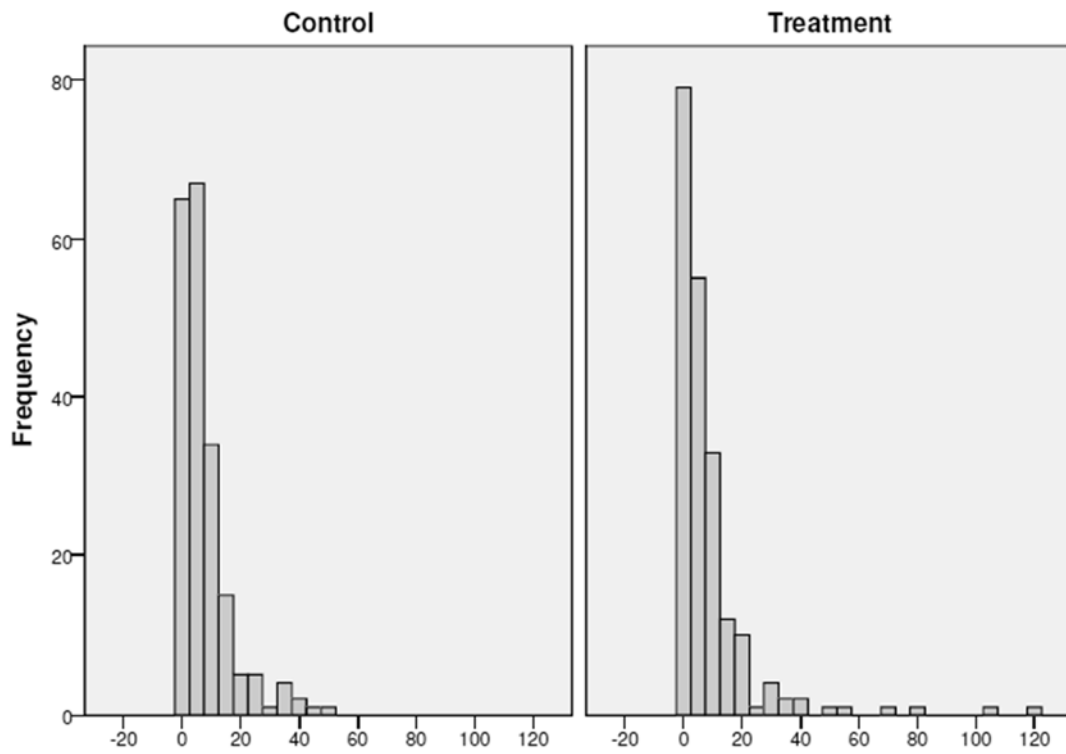
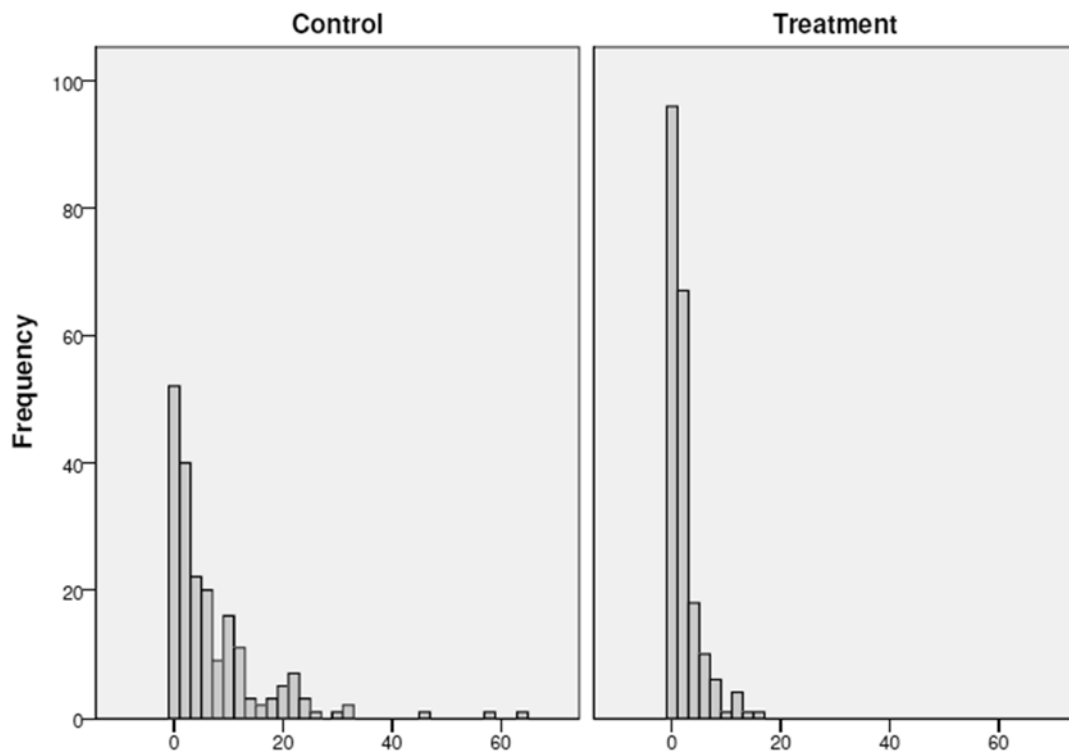


Figure 4.3-2 Histogram of *C. quinquefasciatus* counts from CO₂ light traps in treatment and control



areas.

Figure 4.3-3 - Histogram of *A. notoscriptus* counts from CO₂ light traps in treatment and control areas.

Analysis was conducted for differences between treatment and control groups overall, within areas and among species. The best model fit (QICC = 517.518) was found using the number of gullies containing water, treatment type and area as factors. Treatment was not significantly related to the *C. quinquefasciatus* population. ($p=0.281$). Treatment was significantly related to *A. notoscriptus* numbers ($p= 0.000$, 1df). Three times as many *A. notoscriptus* were found in the untreated area. The estimated marginal mean for the treatment group was 1.86 (95% C.I. 1.43 to 2.42) and 5.88 per trap for the control areas (95% C.I. 3.97 to 8.72). Having 2 gullies containing water within the Voroni segment correlated to a higher catch of *A. notoscriptus* ($p = .003$, 2df). Average catch for traps with 2 wet gullies nearby was 7.96 (95% C.I. 3.91 to 16.19) compared to 1.80 and 2.53 for 0 or 1 drain (95% C.I. 1.20 - 2.70 and 1.96 - 3.27, respectively).

There was a higher population of *A. notoscriptus* in Gooseberry Hill when compared to Maida Vale (4.44 per trap vs 2.47 per trap, respectively, ($p= 0.005$, 95% C.I. 3.37 - 5.84 and 1.63 - 3.73, respectively). The control population in Gooseberry Hill was higher (12.88 per trap, 95% C.I. 9.66 - 17.18) than the treatment population in Gooseberry Hill (1.93, 95% C.I. 1.06 – 2.20) and both treatment and control populations in Maida Vale (2.27, 95% C.I. 1.56 – 3.29 vs 2.69, 95% C.I. 1.45 – 4.97).

Nuisance Level Catches

Three catches were above the Carrieri et al. (2008) level of 40 *Aedes* spp. in a single catch. All were during the control period, 2 in Maida Vale and 1 on Gooseberry Hill. Sixteen catches exceeded the Department of Health (WA) level of 50 mosquitoes. Seven in the treatment areas and nine in the control (11 in Gooseberry Hill and 5 in Maida Vale). Overall 4.0% of catches could be classified as exceeding nuisance levels, 3.4% in treatment and 4.5% in the control periods.

4.4. Mark Release Recapture

Field Results

Released mosquitoes varied in age. Reared mosquitoes were between 2 and 9 days old and were not blood fed. Age and blood-fed status was not determined for the field-caught cohort. The dusting technique impaired a high proportion of mosquitoes and of 491 reared or caught, a

total of 231 (100 pink, 131 orange) were released (47.0%). It was observed that the dusting technique improved as the study progressed with a higher proportion of adults surviving later dusting. Figure 4.4-1 shows mosquitoes that had been heavily dusted, ideally a finer coat would be less likely to impair adults in the field.

Recaptured marked mosquitoes were identified on days 1 (n=5) and day 5 (n=1). All recaptured mosquitoes were female *A. notoscriptus*. All were retrieved from trap 7, 45 m downhill from the release site. No marked mosquitoes were found in the closest trap, 33 m uphill from the release site. There is insufficient data to do a reliable estimate of wild mosquito population numbers.

The ratio of released to recaptured mosquitoes can be used to estimate the wild population numbers. Six *A. notoscriptus* females were recaptured over five days from an estimated 73 released. A total of 17 were caught from wild in the same period giving an approximate 207 wild population using the ratio of released to wild captures. The low number of mosquitoes released in this study would make such an estimate unreliable and should be interpreted with caution.

Survival Analysis

Of the retained sample of adults, 28 were female, 9 were male and 3 could not be identified. Species proportions were reflective of general adult mosquito population: *A. notoscriptus* (n=18), *C. quinquefasciatus* (n=16), *A. alboannulatus* (n=1), *C. australicus* (n=1), *Coq. nr. linnaeus* (n=1), and 3 unidentifiable to species level. Cox regression analysis was conducted for differences between groups, species and gender.

Gender was a significant predictor of survival time ($p=0.000$, 2 df). From this data, the average male lifespan being 20.9% of the average female. Sample size was insufficient to determine differences in survival between dusts types. No significant difference was found for species or treatments. Survival functions for gender and treatment groups are shown in Figure 4.4-2. Sample size was small and overall may not have had sufficient power to detect differences.



Figure 4-4-1 Dusted mosquitoes

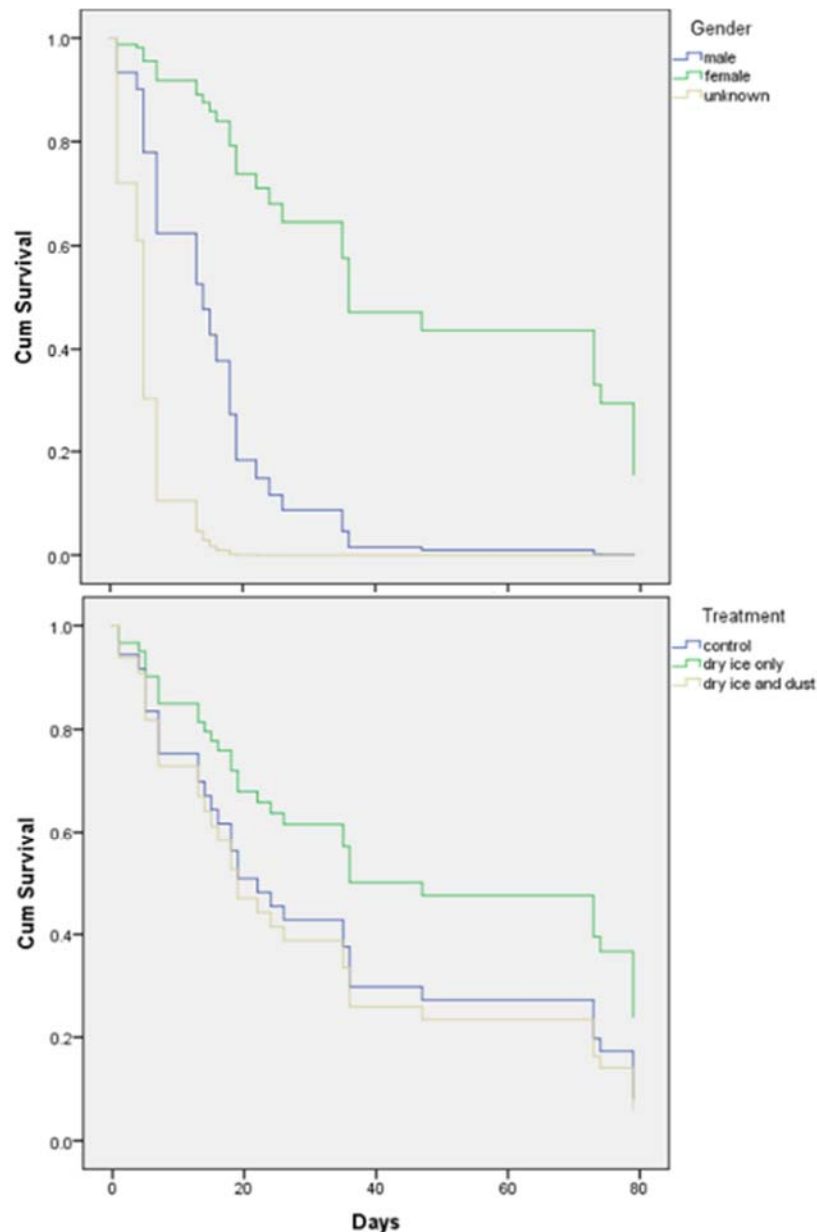


Figure 4-4.2 Survival function for gender and treatment type, control was not treated with dry ice or dust.

4.5. Environmental Sampling

Sampling was conducted on 2 occasions, at the first significant rainfall post treatment. Analysis of the samples was conducted within 1 week of receipt:

- 24 May 2014, 5 samples submitted, all results were below the limit of reporting ($0.1\mu\text{g/L}$ of S-methopene). Samples were taken within 138 days of treatment.
- 20 February 2015, 4 samples, all results were below the limit of reporting ($0.1\mu\text{g/L}$). This sampling was conducted within 65 days of treatment.

5. Discussion

Limitations of study design

The proportion of adult mosquitoes caught in the trap (0.36) was significantly different to previous studies using similar traps. Hamer et al. (2011) collected up to 50% of mosquitoes emerging under the traps when used in gullies (Hamer et al., 2011), and up to 75.5% when used in wetlands (Walton, 2009). Hamer et al. (2011) used conical steel mesh traps in storm-water gullies. The opening of the storm-water gully was not described, and may be the reason for the difference. Walton (2009) used the same pyramidal trap as this study and estimated the proportion at 0.7. He sampled from the surface of a shallow lake, the different environment may account for the large disparity in proportion. This study assumes 100% emergence rates which may lead to underestimated emergence proportions.

An assessment of the proportion of emerging adult mosquitoes being caught in the trap by species was not conducted. Sampling from the storm-water gullies and identification of larva to species level should be done in future studies to address the possibility of differing proportions of *A. notoscriptus* and *C. quinquefasciatus* being caught in the trap.

The mark-release-recapture study suffered from a high mortality rate related to the stunning and dusting procedure. For future studies, less handling is recommended and a dusting technique should be employed that does not rely on stunning with carbon dioxide. Due to the high mortality rate there was an insufficient number of marked mosquitoes released to ensure a robust outcome. For future studies a much larger number of *A. notoscriptus* should be released.

The only six laboratory trials were conducted and this did not allow sufficient sample size to test for a difference in emergence between the gully opening types. For future studies a larger number of laboratory trials, emphasizing the different opening types could be run allowing testing for statistical significance of the means. The emergence proportions found could then be applied to the specific gullies with each opening type allowing finer precision in the results.

5.1. Breeding potential of storm-water gullies

Storm-water gullies have been verified as a productive larval habitat likely to increase the nuisance biting rate for those in close proximity. They are also likely to be contributing to the disease transmission rates for people living, visiting and working in the area. It is not just the number of mosquitoes produced per gully, but also the number of productive gullies, the presence of other larval habitats and the duration of the mosquito breeding season within an area that determines the impact at a community level. Beyond this, the design of the gullies themselves can determine the baseline breeding capabilities of each gully.

Storm-water gullies can breed up to 1600 *C. quinquefasciatus* and 74 *A. notoscriptus* per day. The seasonal (November to March) mean production of *C. quinquefasciatus* was 183 per gully per day. This finding is in agreement with that of Hamer et al. (2011) who found a peak mean of 1,494 *Culex* per m² per day and a seasonal mean of 145 *Culex* per m² per day. These findings are also consistent with other studies conducted in warmer climates, such as 1000 *C.*

quinquefasciatus per day emerging from Tanzanian pit latrines (Curtis and Hawkins, 1982), and seasonal averages of 309 *Culex* per day per m² in Rangoon (now Yangon), Burma (De Meillon et al., 1967), and 58.5 *C. quinquefasciatus* per m² per day from septic tanks in Burma (MacKay et al., 1997 in Hamer et al., 2011).

The mean number of *Culex* per CO₂ trapping night in the control area was 7.4. The storm-water gullies are producing 91 to 183 *Culex* sp. per hectare per day. The CO₂ trapping figure is similar to the range found by Hamer et al. (2011) of 5.6 to 7.7 *Culex* per night, despite having fewer wet storm-water gullies in the area (1, 0.5 and 2 per hectare for Maida Vale, Gooseberry Hill, and Hamer et al. (2011), respectively). This suggests *Culex* are breeding in significant numbers in locations other than the storm-water gullies, most likely in a range of small domestic containers in surrounding residences and other premises. These species are not currently considered an important vector of Ross River virus, therefore further campaigns to locate and eliminate its breeding sites would be to reduce nuisance biting. This could take the form of public education programs and property inspections in response to complaints.

No similar study of production densities was available for a comparison to *A. notoscriptus* catch rates. The marked impact of treatment on their population suggests they are afforded

significant breeding locations by the presence of the gullies, especially where higher vegetation loads, such as grass clipping and leaves, make their way into the sump to provide nutrients for larval development.

Seasonal Peaks and Duration

Significant larval production starts in late October and rapidly increases to a maximum in late spring and early summer. It then reduces substantially until early autumn when it ceases until after winter. *C. quinquefasciatus* emergence rates peak in November and *A. notoscriptus* in December. Substantially higher larval densities were found earlier in the season (Figure 4.2-6) than in previous studies where breeding levels were consistent throughout the season (Knepper et al., 1992), or peaked in the middle of summer (Hamer et al., 2006; Stockwell et al., 2011). This information is important to determine the best commencement time for local mosquito management activities. The study area has a hot dry summer, with the peak of summer less conducive to optimal larval development even for the hardy *C. quinquefasciatus*. The climate of the other studies was warm with continuous rain events, higher humidity and lower maximum temperatures allowing continual higher larval numbers. These results indicate that treatment of roadside gullies should be initiated in early October within the Shire of Kalamunda to maximize their efficacy in killing mosquitoes that breed within these habitats.

As shown in Figure 4.2-6, breeding drops off markedly as humidity drops below 40%. This result is supported by Kay et al. (2000) and Williams and Rau (2011) who found humidity below 40% reduces the ability of *A. notoscriptus* and other species to survive and reproduce. The persistence of breeding beyond January may be due to the humid harborage provided by the gully, allowing resting adults to escape the dryness of summer.

Despite storm-water gullies being a mosquito larval habitat they remain an important infrastructure component of the proper functioning of developments in the urban environment. They are necessary, and installation of gullies with sumps can be minimised but cannot be removed altogether. Therefore, it is important that they are designed to minimise their natural potential for larval development as much as is practicable.

Gully Design and Maintenance

The ability of individual gullies to contribute to nuisance breeding when not treated varies with their construction and placement within the built environment, and with the level of maintenance they receive. Factors examined in this study included; the depth of the sump, sediment depth, the type of opening and the type of surface surrounding the gully.

The depth of the sump gives the gully the ability to hold water. The depth of the water itself influenced breeding, with highest breeding levels at depths of 0.3 m. The depth of the water may provide protection from temperature increases. The mean water temperature was up to 7°C cooler than the maximum air temperature at the peak of summer (Figure 4.2-6). This buffer action prevents water temperatures in deep gullies from exceeding 24°C for any extended period and thereby extending the breeding season, as both *A. notoscriptus* and *C.*

quinquefasciatus have decreased propensity for surviving to emergence above this temperature. Similarly, shallow gully water temperatures would increase more quickly to levels allowing the mosquito season to start earlier in those gullies.

Sediment levels in control samples were also a factor, but not as distinctly. Sediment levels from 0 to 0.2 m correlated to increased breeding, but this dropped off sharply at sediment depths exceeding 0.2 m. This is unlikely to be useful as a practical tool as excessive sediment levels will inhibit the function of the sumps, creating down pipe blockages. It is also difficult to accurately predict sediment build up due to differences in the environment surrounding each gully. For example, a gully near a sandy site or with overhanging trees will fill with sand and debris much more quickly than one surrounded by paved areas. Although the influence of different sediments types was not examined, a highly sandy sediment with little organic matter is unlikely to be as productive as a sediment of decomposing vegetation due to the difference in nutrients available for larvae.

The likely difference between a sandy and organic sediment on larval development is further suggested by the apparent impact that surrounding surface treatment has on larval density. A statistical test could not be conducted as there was a wide variety of surrounding surface treatments. However the two gullies with the highest mean counts over the season both had frequently mown lawns directly adjacent to them with grass and clippings observed in the gully at the time of trap retrieval. The high levels of nutrients in the water from the

decomposing clippings would provide an abundant source of larval food and may be the reason for the extreme numbers. This is supported by Stockwell et al. (2006) who found overall levels of breeding higher in areas with more than 20% surrounding vegetation, and by Baker and Yan (2010) who attributed higher gully breeding in part to levels of organic debris.

The preferred design of gullies in this area would be those with a buffer to surrounding vegetation to minimize larval production. Gullies should not be educted just prior to treatment to allow sufficient build-up of sediment to facilitate action of S-methoprene. Design of gullies alone can impact on base level breeding of mosquitoes, however an understanding of the species diversity of an area, both those breeding locally, and those emigrating from other areas, will further improve the understanding of mosquito dynamics. This in turn will further enhance the effectiveness of control efforts.

5.2. Effectiveness of treatment

Each element of a mosquito control program must be as effective as is reasonably possible to ensure nuisance biting and mosquito borne disease transmission are minimised. Evaluation of the effectiveness has been measured as:

- The effectiveness of the treatment method in controlling the target species, including the current level of resistance in the populations;
- The effect of treatment on the abundance of *C. quinquefasciatus* and *A. notoscriptus* in the area
- The potential for reduced disease transmission within the area; and
- The minimization of S-methoprene, and its breakdown products, outflows into the natural waterways.

Treatment effectiveness and longevity

The mean number of mosquitoes breeding in un-treated gullies was 6 times higher than in treated gullies. Briquet treatment reduced breeding over the season by 89.9% and 91.5% for sink and float treated gullies when compared to control. Maximum control was provided in the first 90 days and declined rapidly over the next 30 days, to zero at day 124 for both application methods.

S-methoprene in the briquet formulation is an effective treatment regardless of application method (float, or non-float). It is likely that as the briquet releases S-methoprene it makes it way slowly to the top layers due to its low solubility and density and stays there unless mixing occurs. This is supported by the findings of Des Lauriers et al. (2006). Mean adjusted mosquito breeding in float treated gullies was 17 mosquitoes per day and 4 in sink treated gullies. Pre-treatment samples of the float-treated group of gullies were higher than the control and sink-treated gullies. The higher post-treatment mean may be an artifact of this higher underlying production rate rather than a difference in effectiveness of the treatment methods. In any case, the difference was not statistically significant, and taken over an entire season has little practical significance in impact on the overall mosquito abundance in the area.

The effectiveness of the treatment decreased markedly after three months, and was well short of the 150 days as stated on the label. The briquets were too small to be detected by day 96 and were no longer providing control by day 125. Field effectiveness of S-methoprene has been shown to be lower than laboratory trials (Knepper et al., 1992) with the difference ostensibly attributable to flushing during rain events (De Lauriers et al., 2006). In the field, S-methoprene was 55% and 89% effective at 105 days, in debris free and debris filled gullies respectively (Baker & Yan, 2010). Their results support this study which has 75% and 63% control (for float and sink treatments, respectively) at day 96 for a mixture of sediment levels.

The briquet has been shown to prevent over 90% of adult emergence cumulatively over 124 days. This is a very high level of control. Prior studies have found cumulative control levels of 69.5% and 70% over 133 and 105 days, respectively (Knepper et al., 1992; Stockwell et al., 2006). The gullies in the prior studies were subject to much more frequent rainfall and milder temperatures than this study. These factors allow more significant mosquito breeding later in the season than this study, where hot dry conditions have curtailed breeding later in the summer. Higher numbers of mosquitoes breeding at the middle and end of the season would result in a lower cumulative effectiveness figure. Additionally, effectiveness was measured in one study by removing larva and their water and keeping for 14 days in the laboratory, rather than in the field, allowing the dissipation of S-methoprene and possibly a higher emergence rate than would have occurred in the field (Stockwell et al., 2006). Overall the environmental conditions in Southwest Western Australia concentrate the bulk of mosquito production early

in the season. This contrasts with most of the previous studies which had been conducted in wetter, milder climates of North America, allowing breeding throughout the season. This difference in breeding dynamics must be considered when designing control programs in similar climates.

Boxmeyer, Leach and Palchick (1997) found levels of S-methoprene degrade linearly and briquet weights degrade logarithmically, but did not analyse or comment on how this correlates with effectiveness. This study suggests that the effectiveness of the briquets does not degrade linearly (Figure 4.2-7). Baker and Yan (2010) assumed a linear degradation in briquet effectiveness, but ceased measurements at day 105, so comparison of effectiveness after this point is not possible. The exact relationship may be negative exponential, or a quadratic or cubic relationship. The nature of the curve is difficult to estimate as the exact number of days of effectiveness is only known to be between 96 and 125 days. Regardless of the exact relationship the effectiveness of the briquets drops rapidly after 3 months of submersion and is nil by month 4. This contrasts with Stockwell et al. (2006), who found partial residual activity after 133 days. Higher average water temperatures in this study may cause more rapid degradation of the briquet decreasing the longevity of the briquet.

The briquets had degraded below a detectable size by day 96. Boxmeyer, Leach and Palchick (1997) estimated that, by weight, nearly a quarter (~8 g) of the briquets should have not dissolved at 120 days, and that overall the briquets should not fully dissolve until day 500. Within their study there was a large range of complete degradation, from 50 days to well over 500 days. They found that briquets which were not continually submerged, took a larger number of submerged days to completely degrade. In this study briquets were almost exclusively submerged, this may explain the more rapid degradation. Future studies could examine the relationship between temperature and degradation time more explicitly.

The high level of effectiveness provided by briquet treatment suggests that resistance has not developed among the target species despite 15 years of continuous use. Resistance was not formally investigated in this study as there are no readily available sources of the local species, not previously exposed to S-methoprene, with which to conduct a comparison. However studies on the exposed population could be done by exposing them to increasing levels of S-methoprene and determining the proportion successfully emerging. Further studies could

assess the level of resistance using bioassays. Despite there being little evidence for the development of resistant populations, it has been shown to develop quickly (Cornel et al., 2002) and it would therefore be prudent to periodically monitor for its development.

Effect on Mosquito Abundance

There was no statistically significant reduction in the numbers of *C. quinquefasciatus* caught by CO₂ light trap in the treatment area, despite the gullies breeding moderate numbers of them throughout the season. As they constitute the majority of mosquitoes in the area, it is not surprising that treatment had little impact on the number of nuisance level catches in the area. This suggests that they are breeding in many other domestic locations as well as the gullies. These are likely to include septic tanks and domestic rainwater tanks. The number of rainwater tanks may continue to increase following water-use reduction campaigns (Trewin, Kay, Darbro & Hurst. 2013). Public awareness of the need to ensure mosquito proof covers are intact in septic tanks and rainwater tanks will be needed to mitigate these sources.

Treatment of storm-water gullies correlated with a 3-fold reduction in numbers of adult female *A. notoscriptus* caught (5.88, control to 1.86, treatment). This effect was even more marked in Gooseberry Hill, showing a 6.5 fold reduction (12.88 to 1.93 per trap). Maida Vale showed only an 18% reduction, however, this area was sampled 124 days post-treatment, when the briquet has been shown to be past its period of effectiveness. In contrast, Gooseberry Hill was sampled 60 days post treatment when effectiveness was above 90%. Traps with two wet gullies in close proximity caught three and four times as many *A. notoscriptus* as traps near only one or no wet drains. This, further supports storm-gullies as being a significant breeding location for this species, extending their season and range. Treatment has an appreciable impact on this species.

Effect on Disease

The most common mosquito borne disease in the Shire of Kalamunda is RRV. The virus has multiple transmission pathways including importation via mosquitoes or human cases, and local transmission via a range of mosquito vectors.

The major inland vector of this disease in Western Australia is *C. annulirostris*. This species is capable of traversing long distances and importing the virus into the area. *Culex annulirostris* does not breed in storm-water gullies, but prefers ponded freshwater (Ritchie et al., 1997). The

CO₂ trapping completed in March did not find them in significant numbers, however previous counts done by the Shire of Kalamunda (Figure 2.1-2) have found them to contribute up to 5% of the catch in December-January. As the ephemeral ground pools diminish over summer the number of *C. annulirostris* in the area drops. Their presence in the early summer coincides with the highest rate of RRV transmission in the area (Table 2.2-1), however the disease is transmitted at lower rates at other times so alternative transmission pathways must be active.

An alternative importation pathway is the movement of viraemic humans. People can import RRV from outside the area, for example, people returning from highly endemic areas in the Southwest of Western Australia. This is considered to be a significant mode of transmission (Russell 2002). Once the virus is present in the area transmission to humans can be by a number of mosquito vectors, including *C. annulirostris* and *A. notoscriptus*.

An average of 33 cases of RRV have occurred each year in the past 5 years (Table 1.1-1). This equates to an annual average of 2672 virus positive (though not symptomatic) people, when using the 1:80 clinical to subclinical infection rate as determined by Kay and Aaskov (1989). This is roughly 1 in 20 people as the population of the Shire of Kalamunda is just over 50,000, and is ample to facilitate person to person transmission via local species. Reducing the abundance of *A. notoscriptus* via treatment of gullies may be sufficient to reduce the transmission of this disease in the local area.

A. notoscriptus is also a vector of this disease. Infection rates of *A. notoscriptus* are similar to *C. annulirostris* (1.7 vs 2.3 per 1000 mosquitoes) and it is known to be an aggressive biter of humans (Ritchie et al., 1997). The high prevalence of *A. notoscriptus* when compared to *C. annulirostris*, coupled with its preference for human blood meals, increases the likelihood that they are a common disease pathway in this area. It has been shown that the interaction of *A. notoscriptus* and *C. annulirostris* is responsible for significant transmission of the disease to occur (Hu et al., 2010). This should be interpreted with caution however, due to the significant difference in species diversity in that study, where *A. notoscriptus* accounted for only 4% of the population. In this study it constitutes over 60% of the mosquito population and this is likely to increase the importance of its role in local RRV transmission.

A note of caution however, the adult trapping for this study was conducted at the end of the

season, presumably after smaller bodies of water suitable for *C. annulirostris* and *A. notoscriptus* breeding had dried out. There may be less of an impact on overall *A. notoscriptus* numbers earlier in the season when there are a larger number of alternate breeding sites available, therefore the influence of treatment on RRV transmission may be limited at this time.

The interaction of *A. notoscriptus* and *C. annulirostris* is likely to significantly increase the rate of transmission of RRV therefore sentinel traps are recommended to be set up in the Shire to see when numbers increase. Once several years of data has been collected, it will be possible to identify a trigger value for *C. annulirostris*, above which virus transmission is likely to increase above a set limit. Regular monitoring for *C. annulirostris* and public information targeted at times of high abundance of this vector may help reduce the number of cases relative to the regional rate.

Effect on Environment

There appears to be no substantial escape of S-methoprene into the natural waterways at the point of discharge from the storm-water gully system. No residual S-methoprene was detected in any of the 9 samples taken over the study period. However, S-methoprene is notoriously difficult to detect (Kuo et al., 2010). If the study is repeated it would be useful to take periodic samples directly from treated gullies concurrent with end of pipe samples, and between rainfall events to allow for a more comprehensive analysis of how detected levels compare with effectiveness.

Despite the difficulty in detecting residual S-methoprene, for the dry climate of Perth, with the potential for a month or longer with no significant rainfall, the large lag time between treatment and flushing and the logarithmic decay of briquet mass, means the initial peak of S-methoprene release can be well past. All together the likelihood of discharge of significant levels into environment is low and likely to be highly sporadic. Ongoing impact on the fauna of the adjacent creeks and wetlands is unlikely.

6. Summary and Program Recommendations

This study has examined the Shire of Kalamunda mosquito control program in the local context, however the applicability of the findings are broad. Many local governments within Southwest Western Australia implement similar storm-water gully control programs, and many also use S-methoprene for mosquito control in wetland and similar environments. These results will be of interest to them and to those in any area with a climate of high heat and low rainfall over summer. The most significant findings include the:

- high productive capacity of storm-water gullies,
- role that storm-water gullies may play in Ross River virus transmission rates,
- effectiveness of S-methoprene when used without a float, and
- sharply decreasing effectiveness of S-methoprene after 90 days.

Floating Emergence Trapping has confirmed that storm-water gullies are capable of producing large numbers of mosquitoes so require control. Results of CO₂ light trapping and Floating Emergence Trapping both indicate that breeding peaks in early November, and tapers off by February and March as hot dry conditions curtail breeding. Summer has traditionally been considered the most productive time for mosquito breeding in local government in Western Australia, however these results show treatments must already be in place by early October. This will require alteration of treatment schedules, associated recruitment of personnel and rescheduling of gully education programs.

Storm-water gullies have been shown to produce two main species; *Culex quinquefasciatus* and *Aedes notoscriptus*. Both have been found to be the most abundant species within the Shire at all times in the season, and are container breeders, reproducing profusely in close contact with human habitation. *A. notoscriptus* is a verified vector of RRV, and has been found to be an important transmitter of this disease. The potential for RRV transmission increases when numbers of *Culex annulirostris* are higher than usual. This occurs in the Shire early in the season when ephemeral ground pool and similar freshwater sites are available in which *C. annulirostris* can breed in large quantities. At other times of the season the numbers of *C. annulirostris* are constrained. Treatment of storm-water gullies with S-methoprene briquets can be correlated with a lower abundance of *A. notoscriptus*, but not *C. quinquefasciatus*. As *A. notoscriptus* is an

important vector of disease, and a notoriously vicious biter of humans, treatment of storm-water gullies to reduce their abundance is warranted to reduce their numbers and potentially reduce the burden of RRV in the area.

As a part of this study it had been proposed that 5 gullies would be physically altered to non-water holding design by filling the water holding section with concrete so that the outlet pipes are flush with the gully floor, or by other agreed method. Gully modification had not been undertaken at the end of the study period due to a delay in the Asset Inspection Program run by the Shire of Kalamunda which was caused by proposed local government amalgamations. It is highly recommended that this portion of the study be undertaken in future.

Treatment of storm-water gullies with a briquet is effective in preventing larval emergence into adult mosquitoes for both common species, regardless of whether a float is used or not. This contrasts with the label specification that the briquet be suspended above sediment. Not having to suspend the briquet removes the need for purchasing and attaching a float, which is a considerable saving in materials and time. Floats are commonly used when applying S-methoprene to wetland areas. It is likely that the equal effectiveness of float and non-float application methods are applicable to this environment, but further studies may be needed for confirmation.

Finally, the results show S-methoprene briquets are highly effective for 3 months and retains some residual effectiveness for a further month, before dropping to zero within 120 days, well short of the 150 day maximum stated on the product label. As the storm-water gully productivity has dropped significantly by this stage in the study area, in other areas with mosquito season exceeding 4 months, and where a high level of mosquito production continues later in the season, reapplication of briquets will be required after 3 or 4 months to sustain a high level of control. The interruption of adult emergence in early October before populations start to significantly increase is likely to reduce the overall mosquito control effort required. This has significant implications for control programs in recruitment and purchase of S-methoprene briquets.

Program Recommendations

The fundamentals of the mosquito control program as implemented by the Shire of Kalamunda have been found to be sound. The program addresses a real mosquito breeding problem with an effective treatment method. However, some improvements and additions to the program are recommended including; timing of treatment application, coordination of gully cleaning programs, ongoing monitoring for priority vectors, and monitoring for evidence of pesticide resistance.

Timing of treatment application

The most significant alteration to the existing program is commencing treatment earlier. Application currently starts in December. The results of this study show that peak breeding has already occurred and the flow of new mosquitoes into the environment is already slowing by this time. This means that breeding is uncontrolled at its most active stage, and a high proportion of the seasons mosquitoes are evading treatment. Application of the S-methoprene briquets should be done in early October. This would give a high level of control until late January and partial control for February. This should provide a sufficient level of overall control as productivity of the storm-water gullies is declining by this time. Also, treating earlier in the season should reduce the overall number of mosquitoes surviving to breed again later in the season.

Coordination of gully cleaning program

The presence of sediment can enhance the effectiveness of S-methoprene by up to 34% (Baker and Yan, 2010). To maximise this enhancement of the treatment program some sediment should be left in place. To achieve this the Shire's gully cleaning program should be timed to avoid cleaning in the two months prior to application and not recommence until late February.

Monitoring for development of resistance

Periodic monitoring for larval resistance to S-methoprene by floating emergence trapping should be included as a part of the program. Resistance can occur quickly and without actively monitoring for it, may not be noticed for some time as overall numbers of mosquito complaints

received by the local authority are low, even in seasons when treatment has not been applied (Shire of Kalamunda – personal communication).

Sentinel trapping for *Culex annulirostris*

The likelihood of transmission of RRV is highest when the number of *Culex annulirostris* increases, therefore it is recommended that a weekly CO₂ light trap sample is taken. When the proportion of this species rises above a trigger value press releases and other forms of public communication can be implemented advising the public of increased mosquito activity and the actions they can take to protect themselves. Promotional materials and information such as those provided by the Department of Health (WA) “Fight the Bite” campaign are readily available.

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8. Appendix 1 – Prolink Briquet label.

Wellmark Logo

READ SAFETY DIRECTIONS BEFORE OPENING OR USING

ProLink™ XR BRIQUETS
MOSQUITO GROWTH REGULATOR

ACTIVE CONSTITUENT: 18 g/kg (S)-METHOPRENE (dry weight basis)

(This product contains water, therefore the weight of the briquet and percent by weight of active constituent will vary with hydration.)

| | | |
|-------|----|-------------|
| GROUP | 7A | INSECTICIDE |
|-------|----|-------------|

A SUSTAINED RELEASE PRODUCT TO PREVENT ADULT MOSQUITO EMERGENCE

BATCH NUMBER:
EXPIRY DATE:
D.O.M.
MADE IN USA

Distributed by:
Pacific Biologics Pty Ltd
35 Beach Street
Kippa Ring QLD 4021
(07) 3283 5077
(8:30 am to 5:30 pm E.S.T. Monday to Friday
www.pacificbiologics.com.au

Net Contents: 8 kg dry weight basis
contains 220 briquets

DIRECTIONS FOR USE: Restraint: DO NOT use in areas grazed by livestock.

| Site | Pest | Rate | Critical Comments |
|---|-----------------------------|---------------------------------|--|
| Dams, storm drains, catch basins, roadside ditches, ornamental ponds and pools, cesspools and septic tanks, sewage treatment settling ponds, abandoned swimming pools, construction and other man-made depressions, ponds and pools in freshwater swamps and marshes, salt marshes, mangrove swamps, woodland pools and flood plains. | <i>Ochlerotatus vigilax</i> | 1 briquet per 20 m ² | Use this rate when water is shallow (<30 cm), clean and where larval mosquito counts are low (<10 per dip). |
| | | 1 briquet per 10 m ² | Use this rate where water is deep (>30 cm) or is rich in organic matter or sediment, or where larval mosquito counts are high (>10 per dip). |
| | All other mosquito species | 1 briquet per 10 m ² | Use 1 briquet per 10 m ² for all other mosquito species. |
| Rain water tanks (including those containing potable water) | All mosquito larvae | 1 briquet per 5,000L water | Retreat rainwater tanks at 6 monthly to yearly intervals. |

NOT TO BE USED FOR ANY PURPOSE, OR IN ANY MANNER, CONTRARY TO THIS LABEL UNLESS AUTHORISED UNDER APPROPRIATE LEGISLATION

Users in NSW must comply with the Protection of the Environment Operations Act, 1997 and its subsequent amendments.

GENERAL INSTRUCTIONS

ProLink™ XR BRIQUETS provide extended mosquito control in chronic, long-term breeding sites by preventing the emergence of adult mosquitoes, including common *Aedes*, *Anopheles*, *Culex* and *Ochlerotatus* spp. Treated larvae continue to develop normally to the pupal stage after which they fail to emerge. (S)-methoprene has no effect on mosquitoes which have reached the pupal or adult stage of development at time of treatment.

Application:

ProLink XR BRIQUETS are designed to release (S)-methoprene over a period up to 150 days in submerged mosquito breeding sites. Soft mud and loose sediment can cover the briquets and inhibit normal dispersion of the active ingredient. In such sites briquets may be placed in mesh bags, tied to stakes, to prevent the briquet sinking into the substrate. This also enables easy monitoring of the rate of breakdown on the briquets. Similarly a briquet suspended in a mesh bag in a water tank can be checked at intervals.

ProLink XR BRIQUETS may not be effective in those situations where the briquets can be removed from the site by flushing action. Briquets should be anchored as described above.

ProLink XR BRIQUETS should be applied before or at the beginning of the mosquito control season. The briquets can be applied prior to flooding or rainfall when sites are dry. Briquets should be placed in the lowest areas of mosquito breeding sites to maintain continuous control as the site alternately floods and dries up. Under normal conditions, one application should last the entire mosquito control season, or at least 150 days, whichever is shorter. Alternate wetting and drying will not reduce the effectiveness of **ProLink XR BRIQUETS**.

Aerial application rates should be frequently checked by comparing amount of product used against area flown during application.

Insecticide Resistance Warning

| GROUP | 7A | INSECTICIDE |
|-------|----|-------------|
|-------|----|-------------|

For insecticide resistance management, **ProLink** is a Group 7A insecticide. Some naturally occurring insect biotypes resistant to **ProLink** and other Group 7A insecticides may exist through normal genetic variability in any insect population. The resistant individuals can eventually dominate the insect population if **ProLink** or other Group 7A insecticides are used repeatedly. The effectiveness of **ProLink** on resistant individuals could be significantly reduced. Since occurrence of resistant individuals is difficult to detect prior to use, Wellmark International accepts no liability for any losses that may result from the failure of **ProLink** to control resistant insects.

STORAGE AND DISPOSAL

Keep out of reach of children. Store in the closed, original container in a dry, cool, well-ventilated area out of direct sunlight.

Triple or preferably pressure rinse container into the treated water. Do not dispose of undiluted chemicals on-site. Break, crush or puncture and bury empty containers in a local authority landfill. If no landfill is available, bury the containers below 500 mm in a disposal pit specifically marked and set up for this purpose clear of waterways, vegetation and tree roots. Empty containers and product should not be burnt.

SAFETY DIRECTIONS

Will irritate the eyes and skin. Avoid contact with eyes and skin. When using the product, wear elbow-length PVC gloves. If dust is present wear faceshield or goggles and disposable dust mask. If product in eyes, wash it out immediately with water. Wash hands after use. After each day's use, wash gloves and faceshield or goggles.

FIRST AID

If poisoning occurs, contact a doctor or Poisons Information Centre (Phone: 131126)

Material Safety Data Sheet

If additional hazard information is required refer to the Material Safety Data Sheet. For a copy phone your distributor.

WARRANTY AND EXCLUSION OF LIABILITY

This product is warranted fit for the purposes specifically recommended by Wellmark International when used strictly as directed on this label. All other warranties and obligations for liabilities, whether expressed or implied by statute or otherwise, are excluded to the full extent that exclusion is permitted by law.

APVMA Approval No. 58061/220/0505

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Made in USA